EXTRACTION, DEGRADATION, AND MICROBIAL RESPIRATION EFFECTS OF MESOTRIONE IN SELECTED TEXAS SOILS

A Dissertation

by

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ABSTRACT

The heavy use of pesticides in agriculture, have encouraged researchers to evaluate their behavior and potential environmental impacts of newer herbicides when applied alone or in combination with other herbicides. Mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1, 3-cyclohexanedione), a pre- and post-emergence herbicide is one of these newer herbicides used to control broadleaf weeds in corn. Assessing the soil behavior of newer herbicides is important to understand the potential impacts on key ecological processes. The primary objectives of this research were to: 1) determine the optimal conditions to extract mesotrione from four soils with varying physical and chemical characteristics using Accelerated Solvent Extraction (ASE); 2) study the effect of atrazine on mesotrione degradation in soil; and 3) determine if mesotrione, mesotrione + atrazine treatments, and application rates had an impact on soil microbial activity (respiration).

In the first experiment, mesotrione recoveries were not significantly different between two tested solvents across the four soils. The 4:1, acetonitrile: 5% acetic acid solvent was selected as the extraction solvent for all subsequent tests. When the three static cycles (1, 2, and 3) were evaluated, mesotrione recoveries were not significantly different between the three static cycles across the four soils. The two static cycles was selected as optimal, resulting in higher recoveries for the four soils. The investigation of extraction temperatures (50°C, 100°C, and 150°C) resulted in no significant differences between temperatures of 50°C and 100°C, and the temperature of 50°C was selected since higher recoveries obtained with that temperature. In the second experiment, the

results demonstrated that mesotrione + atrazine herbicide mixtures have the potential to decrease mesotrione degradation in soils. However, it remained unclear whether the reduced degradation was due to the combined impacts of the herbicides, varying soil characteristics, and/or the soil microbial populations present in each soil. The third experiment resulted in the mesotrione and mesotrione + atrazine treatments that inhibited microbial activity (respiration) only at certain incubation time periods and rates for some soils. Furthermore, the mesotrione treatment was found to also stimulate microbial respiration at the 10X rate in the Orelia soil. Although rates effects on microbial respiration occurred, a trend was not observed in this study.

DEDICATION

To my mother, who has supported every decision without question and loved me unconditionally.

To my loving husband, who instilled in me the inspiration to set high goals and the confidence to achieve them. Thank you for sharing with me many uncertainties, challenges, and sacrifices.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Pesticide persistence is the ability of a chemical to maintain its integrity in the environment over a period of time. It is often expressed in terms of half-life ($t_{1/2}$), which is the length of time required for one-half of the original chemical to degrade. The persistence of a chemical is affected by several degradation processes. The degradation of a chemical in the soil can be influenced by the pesticide's physical and chemical properties, soil pH, soil organic matter, and environmental conditions. If conditions favor rapid degradation, the pesticide may not persist long enough to adequately control the target pest. If the pesticide persists longer in the environment and is not rapidly degraded it has the potential to become mobile, runoff, leach, and contaminate nearby water sources. Studies have already identified the presence of many pesticides in surface and groundwater sources, causing negative impacts to human health and the environment. For this reason, it is important to evaluate the degradation and persistence of newer pesticides that enter the market in order to predict their behavior in soils once applied and to minimize unintended negative consequences.

Literature review

Mesotrione background

Mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione) is a member of the triketone chemical family. The discovery of the triketone herbicides started in 1977 when a scientist with Zeneca Group PLC, a former pharmaceutical

company, observed that there were few weeds growing under the bottlebrush plants (*Callistemon citrinus*). After careful examination, it was discovered that this was due to a natural herbicidal compound found in these plants called leptospermone. A second event took place in 1982, when Zeneca chemists were trying to obtain functional mimics of a compound. Herbicidal activity was expressed in some of the analogues produced, triggering further analogue development. During this process, a 2-chlorobenzoyl analogue was created for another purpose, but it was found to be herbicidal and produced similar effects to leptospermone. Further studies with this compound resulted in the discovery and development of triketone (Mitchell et al. 2001).

Mesotrione was first registered for use in the United States (U.S.) in 2001 under the trade name Callisto®. It is a weak acid that has a pK_a of 3.12 (Mitchell et al. 2001). Mesotrione will dissociate from the molecular form to the anionic form as the pH rises. When the pH is below the pK_a, mesotrione will be found in the undissociated molecular form (with H^+ ion), increasing the ability for mesotrione to adsorb to soil colloids while simultaneously slowing degradation. When the pH is above the pK_a, mesotrione will be found in the dissociated anionic state (without H^+ ion), decreasing soil adsorption, increasing the degradation rate (Dyson et al. 2002).

Mesotrione application rates and weed control

Mesotrione provides pre- and post-emergence control of broadleaf weeds in corn (*Zea mays*). Application rates range from 100 to 225 g ha^{-1} (pre-emergence) and 70 to 150 g ha^{-1} (post-emergence) (Mitchell et al. 2001). Effective mesotrione control has been observed with various weeds including the common cocklebur (*Xanthium strumarium*

L.), Amaranthus species (*Amaranthus spp.*), jimsonweed (*Datura stramonium* L.), velvetleaf (*Abutilon theophrasti* Medicus) and common sunflower (*Helianthus annuus* L.) (Abendroth et al. 2006).

Mesotrione mechanism of action

Mesotrione acts by inhibiting the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, a component of the biosynthetic pathway in plants that converts tyrosine to plastoquinone and α-tocopherol (Mitchell et al. 2001). Plastoquinone is required as a cofactor for the enzyme phytoene desaturase which is used in carotenoid biosynthesis. Carotenoids are required for photosynthesis and protection of the chlorophyll and plant cell membranes during photosynthesis (Cornes 2006). Following treatment, sensitive plants will experience a disruption in carotenoid biosynthesis in the chlorophyll pathway, resulting in a bleaching effect followed by plant necrosis.

Mesotrione metabolites

Two mesotrione biotransformation products have been previously identified including, 4-methylsuflonyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonylbenzoic acid (AMBA) (Alferness and Wiebe 2002). In 2006, Durand et al. (2006) conducted a metabolite profiling study using liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry to investigate the metabolic pathway involved in the biotransformation of mesotrione by the bacterial strain *Bacillus* sp. 3B6.

Mesotrione degrading microorganisms

Several studies have been conducted to identify soil microorganisms capable of

degrading mesotrione in soil. Durand et al. (2006) successfully isolated and characterized a strain of bacteria that biotransformed mesotrione. The strain was isolated from cloud droplets (cloud water) and showed a phylogenetic relationship to the *Bacillus* genus. This study was the first to report a rapid mesotrione biotransformation by a pure bacterial strain.

Later, Batisson et al. (2009) conducted a study where bacteria from mesotrione treated soil cultured in a mineral salt solution supplemented with mesotrione as a sole source of carbon. The bacterial community structure of the enrichment cultures was analyzed by temporal temperature gradient gel electrophoresis (TGGE) which revealed that mesotrione had an impact on the bacterial community structure only when using the highest concentration (100 mg L^{-1}). This study isolated and characterized a mesotrione-degrading *Bacillus* sp. from soil capable of biotransforming mesotrione.

Toxicity of mesotrione to microorganisms

The potential effect of mestorione on specific microorganisms has been investigated. Bonnet et al. (2008) conducted a toxicity assessment of the herbicides sulcotrione and mesotrione towards two reference environmental microorganisms, *Tetrahymena pyriformis* and *Vibrio fischeri*. They also wanted to assess the toxicity of different degradation products. A slight toxic effect was observed on the nonspecific esterase activities of *Tetrahymena puriformis.* The commercial product Callisto® had a greater toxicity than the technical grade formulation. A toxic effect on the metabolism of *Vibrio fischeri* was also observed, again with the greatest toxicity being observed with

the commercial product. Most of the degradation products studied showed a greater toxicity than the parent molecule.

Crouzet et al. (2010) conducted a microcosm study to determine the response of soil microbial communities to mesotrione using pure and commercial formulations applied at three different doses (1X, 10X , and 100X rate). The effects were assessed on the overall microbial activities and prokaryotic cell abundances. When mesotrione was applied at the recommended field rate, no impact was observed on the soil microbial communities. When the doses exceeded the recommended rates, an impact was observed on non-target soil microorganisms, inducing an increase in microbial respiration. They concluded that not seeing a microbial impact at the lower doses did not mean that the bacterial and fungal communities remained undisturbed but that further studies on specific microbial groups are necessary to further assess the microbiological impact of mesotrione.

Mesotrione degradation and mobility studies

Degradation and mobility studies involving mesotrione have been conducted to give insight into the pesticide's persistence in the environment. Rouchaud et al. (2000) evaluated the dissipation of mesotrione in soils of corn crops possessing different textures but similar pH and organic matter content. This study gave some insight relating to the mobility of mesotrione in soil. They found that more than 90% of mesotrione was in the 0- to 10-cm soil layer during three months after application with no mesotrione residues being detected in the 15-to 20-cm soil layer. Soil half-lives were 50 days (loam soil), 41 days (sandy loam and clay soils), and 34 days (sand soil). This study suggests

that the mobility of mesotrione remains in the top soil layers and does not move to deeper depths.

The same research group, Rouchaud et al. (2001), followed up these results by evaluating the mobility of mesotrione in the 0- to 20-cm surface soil layer of crop soils with samples being taken at 7 depths from surface soil down to 10 cm. The soils evaluated had different textures and fertilization treatments. They found that during the first month after treatment, mesotrione remained in the 0- to 2-cm surface soil layer in the clay, loam, and sandy loam soils. In the sandy soil, mesotrione moved downward uniformly. Mesotrione was again not detected in the 15- to 20-cm soil layer for the loam and sandy loam soils but it was detected for the sand and clay soils. Through this study, it was determined that a combination of low soil mobility, depth of penetration, and the rate of soil degradation of this herbicide could explain the lack of movement of mesotrione towards the lower soil layers of field crops.

Other studies have also evaluated the behavior of mesotrione in soils. Dyson et al. (2002) evaluated the adsorption and degradation of mesotrione in 15 different soils from Europe and the U.S. The goal was to understand the influence of soil properties that covered a range of soil pH values, textures, and organic carbon contents. The mesotrione half-lives ranged from 4.5 to 32 days and they found an inverse correlation with increasing soil pH, with half-life. They concluded that mesotrione adsorption was related to soil pH and (to a lesser extent) organic carbon. Chaabane et al. (2008) conducted a degradation study and related to sorption processes of mesotrione and another triketone herbicide, sulcotrione, in two soils. For mesotrione, half-lives for the

two soils varied between 5 and 34 days. This study suggested that the pH and organic matter of each soil influenced the degradation process. Researchers concluded that mesotrione had moderate adsorption, increasing with the clay content of the soil.

Atrazine background

Atrazine use was first registered for use in the U.S. in 1958. Since then it has become a highly used herbicide (Sass and Colangelo 2006). Most of the atrazine usage takes part mostly in the Midwestern part of the U.S. in corn (Solomon et al. 2008). Atrazine is a weak base that has a pK_a of 1.7 and is predominately protonated at soil pH levels lower than the pK_a increasing sorption which is attributed to the formation of the triazine cation (Oliveira et al. 2001). Concerns over its persistence in the environment and entry into groundwater and aquatic environments have surrounded this herbicide (Graymore et al. 2001).

Significant atrazine or metabolite concentrations have been reported in surface and groundwater sources. Studies have been carried out since atrazine was first registered for use to investigate the negative environmental and health impacts associated with its use. Several studies include, Frank and Sirons (1979) where they found that between May 1975 and April 1977, atrazine and its metabolite desethylatrazine were detected in 80% of the 11 streams being evaluated in Ontario Canada. Glotfelty et al. (1984) evaluated atrazine and simazine movement to Wye River Estuary in a three-year project. They found that the total amount of herbicide reaching the estuary depended on the quantity and timing of runoff in respect to application dates. Pionke and Glotfelty (1990) conducted a study in groundwaters from an agricultural

watershed to determine if atrazine and its metabolites were present. They found that atrazine and two metabolites were found in most groundwaters including deep wells, a spring, and groundwaters about to become streamflow. Another study conducted by Koplin et al. (1998) evaluated the occurrence of pesticides in shallow groundwater of the U.S. and found that atrazine was the compound that was most detected.

Atrazine has also been associated with negative impacts to animals and humans. Human exposure to atrazine could occur through exposure while farming and manufacturing or through contaminated drinking water. Leeuwen et al. (1999) conducted a study where existing data was obtained on the incidence of specific types of cancers, contaminated drinking water with atrazine and nitrate, and related agricultural practices in areas in Ontario. They found that atrazine contamination levels were positively associated with stomach cancer incidence.

Several studies have related atrazine exposure to negative impacts to amphibians. Hayes et al. (2002) conducted a study where they found that after exposing frogs to atrazine at low ecologically relevant doses it caused hermaphroditism and demasculinized male frogs. In further studies, Hayes et al. (2010), evaluated what atrazine exposure could cause on adult amphibians. They found that atrazine exposed males were both demasculinized (chemically castrated) and completely feminized as adults, creating the potential for amphibian population declines. Controversy surrounds the findings obtained by Hayes et al. research team, and in response, Syngenta with its own panel of scientists (EcoRisk) have produced several studies to refute these findings (Deb 2006). Due to these conflicting and controversial findings, the U.S. and the

European Union have taken different approaches towards the use of atrazine. In European nations, including France, Germany, Italy, and Sweden atrazine has been banned because of its persistence in groundwater and has been replaced with mesotrione while atrazine is still labeled for use in the U.S (Ackerman, 2007).

Atrazine application rates and weed control

Atrazine provides early pre-plant, pre- and post-emergence control of broadleaf weeds in corn (*Zea mays*) but is also used in sorghum, sugarcane, and other crops (Solomon et al. 1996). Application rates range from 0.5 to 3.36 kg ha^{-1} (pre-emergence) in fallow, 1.1 to 2.2 kg ha^{-1} (post-emergence) in corn and sorghum, and 0.45 to 4.5 kg ha^{-1} (pre- or post-emergence) in sugarcane. Effective control using atrazine has been observed in weeds including pigweed, morningglory, jimsonweed, wild buckwheat, mustard, ragweed, smartweed, cocklebur, and also certain grass weeds such as barnyardgrass and foxtail (Senseman et al. 2007).

Atrazine mechanism of action

Atrazine acts by inhibiting photosynthesis at photosystem II (PS II). Atrazine inhibits photosynthesis by binding to the Q_B-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes. This would block electron transport from Q_A to Q_B and stop CO_2 fixation and production of ATP and $NADPH_2$ which are needed for plant growth (Senseman et al. 2007). Following treatment, sensitive plants will experience interveinal chlorosis of the leaves and yellowing of the margins.

Atrazine metabolites

Deethylatrazine (DEA), deisopropylatrazine (DIA), and hydroxyatrazine (HA) diaminochlorotriazine (DACT), and two dealkylated hydroxyatrazines desethylhydroxyatrazine (DEHA), and desisopropylhydroxyatrazine (DIHA) have been identified as atrazine transformation products (Solomon et al. 2008).

Atrazine degrading microorganisms

Atrazine has been found to be degraded by fungi and bacteria. Mougin et al. (1994) found that biotransformation of atrazine was possible by the white rot fungus *Phanerochaete chrysosporium*. This organism demonstrated a 48% decrease of the initial herbicide concentration in growth medium within the first 4 days of incubation. Another fungus capable of metabolizing atrazine is the fungus *Pleurotus pulmonarius* (Masaphy et al. 1993). Atrazine has also been found to degrade biologically by variety of bacteria (Behki and Khan 1986; Behki et al. 1993; Fadullon et al. 1998; Nagy et al. 1995).

Rapid degradation or enhanced degradation of atrazine in several soils has been reported and has been related to repeated treatments of atrazine (Barriuso and Houot 1996; Pussemier et al. 1997; Yassir et al. 1999). Pure strains of *Pseudomonas* sp. ADP and *Pseudaminobacter* sp. have been able to completely mineralize atrazine to carbon dioxide and ammonia (Mandelbaum et al. 1995; Topp et al. 2000). For *Pseudomonas* sp. strain ADP, the genes for atrazine degradation (atzABC) have been characterized to encode three hydrolases which transform atrazine into cyanuric acid (da Souza et al. 1995; da Souza et al. 1996; Boundy-Mills et al. 1997).

Toxicity of atrazine to microorganisms

The potential effect of atrazine to specific microorganisms has been investigated. Most toxicity studies involving atrazine investigate the effects to aquatic microorganisms. Most studies have focused on short-term growth inhibition due to atrazine exposure on algae species. Abou-Waly et al. (1991) evaluated the toxicity of atrazine and hexazinone to *Anabaena flos-aquae* and *Selenastrium capricornutum*. They found that *S. capricornutum* was more susceptible to hexazinone than atrazine and *A. flos-aquae* was more susceptible to atrazine. Kirby and Sheahan (1994) compared the toxicity of three herbicides atrazine, isoproturon, and mecoprop to a freshwater green algae *Scenedesmus subspicatus* and the common duckweed *Lemna minor*. They found that atrazine and isoproturon were two times more toxic to *Scenedesmus subspicatus* than to *Lemna minor*. The opposite has also been observed where atrazine has been found to stimulate microbial activity more when combined with another herbicide, glyphosate (Haney et al. 2002).

Atrazine degradation and mobility studies

Degradation and mobility studies involving atrazine have been conducted to give insight to the pesticide's persistence in the environment. Most degradation studies with atrazine have focused on enhanced degradation, as previously mentioned. Atrazine is considered to be persistent in the environment, with an average field half-life of 60 days (Wauchope et al. 1992). Atrazine's increased persistence in the environment increases the potential for the herbicide to leach and reach ground and surface water sources.

Bowman (1989) evaluated the mobility and persistence of the herbicides atrazine, metolachlor and terbuthylazine in Plainfield sand. Researchers noted the three herbicides exhibited limited movement in light textured Plainfield sand cores under moderate rainfall. Furthermore, Hall and Hartwig (1978) evaluated atrazine mobility in two soils under conventional tillage and determined that application of atrazine to fine-textured, conventionally tilled soils would not seriously affect ground water supplies through contamination.

Herbicide mixtures of mesotrione and atrazine

Several researchers have found that combinations of mesotrione and atrazine treatment increase herbicidal effects and better control specific weed species by combining two modes of action. Armel et al. (2005) conducted a study to evaluate if mesotrione alone or in mixtures with low rates of atrazine would control Canada thistle (*Cirsium arvense*). This research group found that in field trials mesotrione applied alone did not adequately control Canada thistle, but smaller plants that were in the rosette stage of growth were more susceptible to the herbicide than the ones that were in the bolting stage. When mesotrione was added in combination with atrazine, the control of Canada thistle was improved. Greenhouse studies conducted by this group found that the combination of both herbicides reduced Canada thistle re-growth more than when mesotrione was applied alone. In addition, they observed that the combination of both herbicides increased the rate of tissue necrosis than what would be observed when mesotrione is applied alone. They speculated that the increased control of the herbicide

mixtures was most likely due to the interrelationship between the modes of action of mesotrione and atrazine.

Abendroth et al. (2006), conducted field and greenhouse studies to evaluate the plant responses to combinations of mesotrione and photosystem II inhibitors. They found that all three weed species demonstrated greater leaf necrosis when mesotrione was combined with a photosystem II inhibitor than when it was tested alone.

Creech et al. (2004) investigated the photosynthetic and growth responses of *Zea mays* and four weed species when treated with mesotrione and atrazine. They found that the plants treated with the combination of mesotrione and atrazine had significantly reduced photosynthesis when compared to the controls within 1 day for the five species studied. The combination of the two herbicides suppressed photosynthesis of all species through day 14 except *Zea mays*. They suggested that the improved weed control could be attributed to a joint consequence of damage to carotenoid biosynthesis caused by mesotrione and an influx of active oxygen species caused by atrazine. They explained that the combination of more displaced photochemical energy and less means of quenching that energy could be the reason for increased herbicidal activity.

Extraction techniques

Extraction techniques are integral in the analysis of pesticides and pesticide residues, allowing for the analyst to extract the analyte of interest for further analysis. In the last few years, attempts have been made to improve extraction techniques to reduce the volume of extraction solvent (waste) needed and improve sample extraction time. More traditional extraction techniques include Soxhlet extraction and ultrasonic solvent

extraction. Some of the newer techniques including microwave assisted solvent extraction and pressurized liquid extraction also known as ASE extraction, have replaced traditional methods. In comparison studies, traditional methods were associated with not only producing a substantial amount of solvent waste, but also with being time and labor intensive when compared to ASE techniques which can also be readily automated (Conte et al. 1997; Giergielewicz-Mozajska et al. 2001). Table 1 presents a comparison of ASE techniques with other extraction methods. Traditional methods of extraction could take up to 48 hrs per sample while ASE methods can take only to 12 to 18 minutes per sample (Giergielewicz-Mozajska et al. 2001), saving extraction time. In the case of soil, the ASE extraction process consists of preparing the soil sample by drying it, homogenizing it and sieving it prior to the extraction (Richer et al. 1996). If moisture is present, the sample is mixed with a drying agent such as diatomaceous earth. The drying agent also serves as a dispersing agent and minimizes dead volume in the cell which could cause soil compaction and will provide recovery mistakes in the results. The sample is then added to the stainless steel extraction cell (11-, 22-, or 33-ml cells) then loaded to an oven set at a prescribed temperature. After the chosen time, the solvent is added into the cell and the extraction process begins. The static process begins when the cell is heated at the chosen temperature, where the analyte is isolated from the sample under stable static conditions. This static cycle can be repeated as many times as appropriate to achieve acceptable results. At the end of each extraction, the needle is rinsed with fresh solvent and the entire system is purged with nitrogen to minimize contamination between samples and prepare the system for the next extraction.

Table 1 Comparison of commonly used extraction techniques.

	Extraction Method			
Characteristic	Accelerated solvent extraction	Soxhlet	Ultrasonic solvent extraction	Microwave assisted solvent extraction
Description of method	Sample is enclosed in a sample cartridge that is filled with solvent under high temperature and high pressure[a]	The sample is extracted by adding to thimble-holder and gradually filled with condensated fresh solvent from a distillation flask.[c]	Sonication provides extraction between the solid and solvent. The sample is extracted by adding to an ultrasonic bath.[e]	Microwave used to heat the sample with the solvent in a closed or opened system.[b]
Reported extraction time	12 min[bd]; 12 to 18 min[g]	24 hrs[b]; 4 to 48 hrs[g]	15 min[g]	20 min (atmospheric) and 5 min (pressurized)[b]; 30 min to 1 hr[g]
Reported amounts of solvent used	25 ml[b]; 20 ml[e]; 15 to 40 ml[g]	150 ml[b]; 150 to 500 ml[g]	20 ml[f];100 to 300 ml[g]	40 ml (pressurized) and 70 ml (atmospheric pressure)[b]; 10 to 15 ml[g]
Advantages	Fully automated system, ease of instrument use; short extraction time[a]	Samples are repeatedly in contact with fresh solvent helping displace transfer equilibrium; High temperature maintained[c]	No complex laboratory equipment needed; ease of use	Short extraction time; low amounts of solvent used
Disadvantages	High initial cost of equipment and replacement parts	High extraction time; large amounts of solvent used[c]	Labor intensive; not automated	Moderate amount of solvent used

[a]Richter et al. (1996); [b]Saim et al. (1997); [c]Luque de Castro and Garcia-Ayuso (1998); [d]Richter et al. (2007); [e]Poole et al. (1990); [f]Babic et al. (1998); [g]Giergielewicz-Mozajska et al (2001).

CHAPTER II

ACCELERATED SOLVENT EXTRACTION OF THE HERBICIDE MESOTRIONE FROM SOILS

Introduction

Pesticides have played a pivotal role in agriculture, minimizing invasive weeds, and problem pests. In an attempt to reduce potential environmental and health impacts that have been associated with the use of these chemicals, pesticides are being developed to possess properties that could help minimize these risks. One of these newer pesticides is mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), which is used for pre- and post-emergence control of broadleaf weeds in corn and requires lower application rates than traditional herbicides (Mitchell et al. 2001). The lower application rates aid in minimizing environmental impacts. Mesotrione has gained widespread use as a replacement for atrazine (6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) in European countries where the use of atrazine has been banned (Durand et al. 2006; Swanton et al. 2007; Crouzet et al. 2010; Crouzet et al. 2013) and in the U.S., it is used alone and in combination with atrazine (Armel et al. 2003; Creech et al. 2004; Armel et al. 2005).

It is important to assess the soil behavior of newer herbicides such as mesotrione and the potential impacts they could have on key ecological processes. To do so, analytical techniques are needed, consisting of sample preparation and extraction steps to allow for quantification of the chemical of interest. Current methods to extract mesotrione from soil include solid-phase extraction (Chaabane et al. 2008; Barchanska

et al. 2012) and solvent-shake extraction (Crouzet et al. 2013). There are no published methods to extract mesotrione from soil using accelerated solvent extraction (ASE). ASE is a technique that can significantly improve sample analysis by reducing extraction time while utilizing less solvent and obtaining extraction efficiencies equivalent to or even higher than conventional techniques (Richter et al. 1996; Gan et al. 1999) like solvent-shake extraction or Soxhlet extraction. With ASE techniques, the sample is added to an extraction cell and is then exposed to high temperature and pressure to obtain a solution containing the analyte of interest in a collection vial that is analyzed to determine the concentration of the chemical. The objective of this study was to determine the optimal conditions to extract the herbicide mesotrione by evaluating the influence of extraction solvent, static cycles, and temperature on extraction efficiency from four soils with varying physical and chemical characteristics.

Materials and methods

Chemicals, reagents, and standards

Atrazine, purity 98.8% purchased from Sigma-Aldrich (St. Louis, MO) and mesotrione, purity 99.9% purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile purchased from VWR (Radnor, PA*). Hydromatrix®,* Diatomaceous earth used as a dispersing agent purchased from VWR (Radnor, PA). Formic acid (88%) purchased from Mallinckrodt Chemicals (Mallinckrodt Baker Inc., Phillipsburg, NJ). Individual stock standard solutions were prepared for mesotrione (500 $\mu g\ ml^{-1}$) in acetonitrile and stored at 4°C until use. Working standard solutions of appropriate concentrations were prepared by diluting the stock standard solutions.

General materials and instrumentation

Materials included ASE glass fiber 19.8-mm (Dionex Corp., Sunnyvale, CA), disposable 3-ml plastic syringes with Luer-Lok™ tip (BD, Franklin Lakes, NJ), Millipore™ Durapore® 0.45-mm membrane filters (EMD Millipore Corp., Billerica, MA), and clear 1-ml glass shell vials with polyethylene snap caps (Waters Corp., Milford, MA).

ASE was performed with a Dionex ASE 200 extraction system equipped with 22-ml stainless steel extraction cells and 60-ml collection vials (Dionex Corp., Sunnyvale, CA). Liquid chromatography was performed with a Waters photodiode array system comprised of a Model 616 pump, a Model 717 autosampler, a Model 600S controller equipped with a Model 996 photodiode array detector (Waters Corp., Milford, MA). The analytical column used was a Symmetry Shield RP8, 3.5 µm, C8, 2.1 x 150-mm column (Waters Corp., Milford, MA).

Soil collection and characterization

The soils used in this study include a Weswood clay loam (fine-silty, mixed superactive, thermic Udifluventic Haplustepts), Cameron silty clay (clayey over loamy, mixed, active, hyperthermic Vertic Haplustolls), Orelia sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Argiustrolls), and a Darco loamy sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults). Soils were collected from the surface horizon (0 to 15 cm), brought to the laboratory, air-dried, and then passed through a 2-mm sieve for removal of particles and non-decomposed plant residues. A representative sub-sample of each soil was submitted to the Texas A&M AgriLife

Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas for analysis.

Soil preparation and fortification

Ten gram portions of soil were weighed into 50-ml glass beakers. All samples were re-wetted to bring the soil moisture to 20% (w/w, dry weight basis). Mesotrione in 1 ml acetonitrile was added to the soil samples in glass beakers to obtain a mesotrione concentration of 100 μg ml^{-1} (10 μg g $soil^{-1}$). The herbicide rate was based on recommended application rates and adjusted by an effective interaction depth of 5 cm Mobility studies of mesotrione in soil have demonstrated that the greatest concentration of mesotrione remained in the upper 2 to 4 cm of soil 2.5 months after its application (Rouchaud et al. 2001). This adjustment permits for a more realistic concentration estimate of the herbicide in the soil. After herbicide application, mesotrione was allowed to equilibrate in the soil for 20 min before a 2-g portion of Hydromatrix® was added. The Hydromatrix® served to absorb the moisture and facilitate the complete removal of the sample from the glass beaker. Samples were mixed and transferred to 22-ml ASE extraction cells assembled with a glass fiber filter at the bottom. Non-fortified samples were included in all experiments.

Preparation of stock and working solutions

Mesotrione stock solutions were prepared by dissolving the analytical-grade herbicide in acetonitrile to give a concentration of 500 μg ml^{-1} and were stored at 4°C. The stock solution was brought to room temperature before use. Individual standard working solutions were prepared by diluting the stock solution.

Selection of extraction solvent, static cycles, and temperature

The effect of solvent, static cycle, and temperature on herbicide recovery was evaluated using the four soils. Optimum extraction conditions for mesotrione using ASE methods were evaluated in three studies. The first study evaluated two solvents (1:1, acetonitrile:5% acetic acid; 4:1, acetonitrile:5% acetic acid to determine the most efficient solvent to extract mesotrione from the four soils. In the second study, the most efficient static cycle for mesotrione extractions of the four soils was determined by evaluating 1,2, or 3 static cycles. The third study evaluated the optimum extraction temperature (50°C, 100°C, and 150°C) for extracting mesotrione from the four soils. In all experiments, before the solvent was added, the cells were preheated for 2 min. After the solvent was added, the cells were heated for 5 min. The static cycle was 5 min, where samples were held at the desired temperature and pressure. The cells were then flushed with fresh solvent equal to 60% of the cell volume. The solvent was then purged from the cells by a stream of nitrogen gas for 120 sec and expelled into the respective collection vial. One rinse was included between each extraction for all experiments.

High performance liquid chromatography – photodiode array (HPLC-PDA) analysis

Following sample extraction, each sample was brought up to a final extraction volume of 40, 43, and 45 ml, for the solvent, static cycle, and temperature studies, respectively. Differences in final extraction volume, was due to differences in parameters used. A 1-ml aliquot of each sample was transferred to a 3-ml syringe attached to a 25-mm GHP 0.45-mm acrodisc syringe filter and filtered into 1-ml clear glass shell vials with polyethylene snap caps for HPLC analysis. A Symmetry Shield

RP8, 3.5-mm, C8, 2.1 x 150-mm column (Waters Corp., Milford, MA) was equilibrated with the mobile phase for 1 hr prior to analysis. The instrument parameters included an injection volume of 10 µl and a flow rate of 0.2 ml min^{-1}. The samples were quantified at 270 nm using a photodiode array detector (Halle et al. 2010).

Mobile phase

The mobile phase consisted of 35% acetonitrile, 64.5% water, and 0.5% formic acid. The mobile phase was filtered and degassed through a Millipore filter (0.45-mm) under vacuum.

Statistical analyses

This study was conducted as a completely randomized design consisting of 3 replicates per treatment and the experiment being repeated twice. Variances were tested for homogeneity using the Levene's test and the comparison of group means within each incubation day were analyzed using Fisher's Least Significant Difference (LSD) test at the 5% level of significance (Benedetti et al. 1997) using Statistical Analysis Systems Version 9.3 (SAS Institute, Inc., Cary, NC).

Calibration curve

The calibration curves were plotted by peak area versus concentration of mesotrione. The appropriate concentrations consisting of 0.5, 1, 3, 5, and 10 mg ml^{-1} allowed the construction of a calibration curve. The linear regression equations were calculated with $y = mx + b$, where x was concentration and y the peak areas. The linearity was established by the coefficient of determination (R^2).

Method validation

The accuracy of the method developed was assessed by a recovery test. It was conducted by adding a known amount of mesotrione standards to air-dried samples of each soil (10 g) in 50-ml glass beakers. All samples were rewetted to bring the soil moisture to 20% (w/w, dry weight basis). Mesotrione in 1 ml acetonitrile was added to the soil samples in the glass beakers to obtain mesotrione concentrations of 8, 10, 12, 14, and 16 μg g^{-1}. After application, mesotrione was allowed to equilibrate in the glass beakers for 20 min before a 2-g portion of Hydromatrix® (VWR, Radnor, PA), was added. The Hydromatrix® was added to absorb the moisture and to facilitate the complete removal of the sample from the glass beaker. Samples were mixed and transferred to ASE extraction cells (22-ml) assembled with a glass fiber filter at the bottom. Non-fortified samples were included in all experiments. Samples were extracted with a 4:1, acetonitrile:5% acetic acid solution at 50°C with 2 static cycles. The experiment was performed twice.

Samples were analyzed by HPLC at 270 nm. The injection volume was 10 ml, and the flow rate was 0.2 ml min^{-1}. The mobile phase consisted of 35% acetonitrile, 64.5% water, and 0.5% formic acid. The mobile phase was filtered and degassed through a Millipore filter (0.45-mm) under vacuum.

The experimental design was set up in a completely randomized design with all treatments being replicated three times. The data was analyzed with the SAS statistical system 9.3 (SAS Institute, Cary, NC, USA). The homogeneity of variances was tested

using the Levene's test and the comparison of grouping means was analyzed using the Fisher's LSD test. The difference of significance was determined at 5% level.

Precision

The precision of the intra-day extractions and inter-day extractions were evaluated by repeated injections. The intra-day experiment was done by extracting six replicates of a 0.5 $\mu g\ g^{-1}$ concentration for a day. The inter-day variability was determined by three injections for three days for concentrations of 1, 3 and 5 mg ml^{-1}.

Specificity

The specificity of the method was obtained by extracting a blank sample and a fortified sample. The specificity was used to verify that the endogenous co-eluting components did not interfere with other constituents in the sample.

Calibration curve

The calibration curves were plotted by peak area versus concentration of mesotrione. The appropriate working solutions of concentrations consisting of 0.5, 1, 3, 5, and 10 mg ml^{-1} allowed the construction of a calibration curve. The linear regression equations were calculated with $y = mx + b$, where x was concentration and y the peak areas. The linearity was established by the coefficient of determination (R^2).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD was defined as the lowest concentration of sample determined by the analytical method to obtain the ratio of signal-to-noise of 3:1. For the LOD, the lowest concentration of the herbicide that could be detected but not necessarily quantified was identified. LOQ was defined as the lowest concentration of the compound that was

determined by injecting the known concentration of the diluted standards until the signal-to-noise ratio reached the ratio of 10:1. For the LOQ, the lowest concentration of the herbicide that could be determined with acceptable precision was identified.

Results and discussion

Soil characteristics

Soil characteristics of the four soils used in this study are presented in Table 2. The Cameron, Orelia, Weswood, and Darco soils used were identified as having a texture class of clay, sandy clay loam, sandy loam, and loamy sand, respectively. The Cameron soil has the highest percent clay (42%) and organic matter (1.94%). The Orelia soil had the second highest percent clay (34%) and organic matter (1.58%). The Weswood soil had the third highest percent clay (18%) but the lowest organic matter (0.85%) while the Darco soil had the lowest percent clay (5%) but more organic matter (1.20%) than the Weswood soil. The Cameron and Weswood soils had the highest pH (8.1) followed by the Orelia (7.9) and Darco (6) soils. Preliminary studies were conducted to determine if mesotrione was present in the collected soil samples. No mesotrione residues were detected in untreated soil samples (data not shown).

Selection of extraction solvents

To determine the most efficient solvent for optimum extraction of mesotrione from the four soils, two solvents (1:1, acetonitrile:5% acetic acid; 4:1, acetonitrile:5% acetic acid) were evaluated. The results indicate that mesotrione recoveries were not significantly different between the two solvents across the four soils (Fig. 1). The 4:1, acetonitrile:5% acetic acid solvent was selected for subsequent tests.

Table 2 Selected characteristics of soils used in this study[a].

Parameters	Soil characterization			
Soils collected	Weslaco, TX	Corpus Christi, TX	College Station, TX	Overton, TX
Soil series name	Cameron	Orelia	Weswood	Darco
Texture class[b]	C	SCL	SL	LS
Sand, %	43	50	25	88
Silt, %	15	16	57	7
Clay, %	42	34	18	5
Organic matter, %	1.94	1.58	0.85	1.20
pH	8.1	7.9	8.1	6.0

[a]Samples were analyzed by the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas.
[b]C, clay; SCL, sandy clay loam; SL, silt loam; LS, loamy sand.

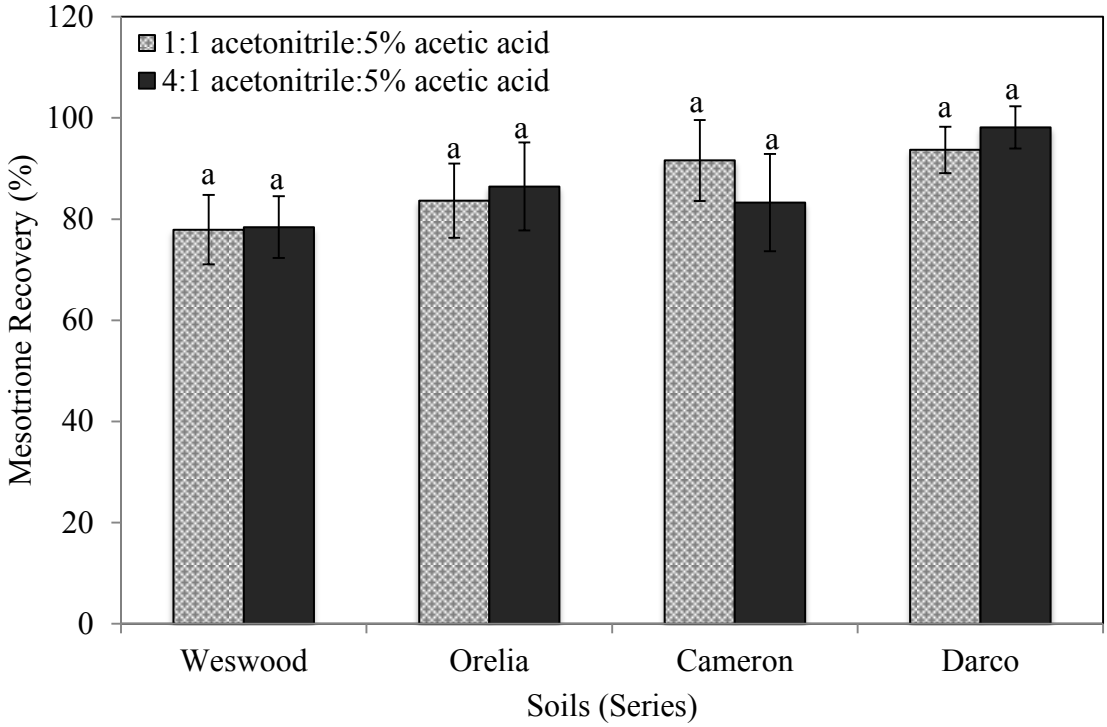

Fig. 1 Influence of two solvents, 1:1 acetonitrile:5% acetic acid and 4:1 acetonitrile:5% acetic acid, on mesotrione recovery from four soils. Two static cycles and a temperature of 50°C were used. Error bars represent standard error of the mean. Different letters denote significant differences ($P \leq 0.05$) for each soil. The figure represents statistical differences between the two solvents for each soil.

Although mesotrione recovery was not significantly different with each of the two solvents across the four soils (Fig. 2), recoveries generally demonstrated a pattern of increasing mesotrione recoveries with increasing % sand in the soils (Weswood < Cameron < Orelia < Darco). Sandy soils have a lower adsorptive capacity than soils with higher clay content, which can explain why mesotrione recoveries increased with those soils that contained a higher sand content. It has been previously observed that mesotrione mobility is increased in sandy textured soils (Rouchaud et al. 2001).

Selection of extraction cycles

To determine the most efficient cycle for optimum extraction of mesotrione from the four soils, three static cycles (1, 2, and 3) were evaluated using the previously identified optimal extraction solvent of 4:1, acetonitrile:5% acetic acid. The results indicate that mesotrione recoveries were not significantly different between the three static cycles across the four soils (Fig. 3). On the basis of mesotrione recovery, it was found that two static cycles were efficient cycles for optimum extraction of mesotrione from the four soils, demonstrating higher recoveries in the four soils. Significant statistical differences were observed in all three static cycles for the Darco (Fig. 4), resulting in higher mesotrione recoveries for this soil, as compared to the three other soils. This was expected, since this soil had the greatest percentage of sand and mesotrione adsorption would be low, allowing for higher recoveries.

Selection of extraction temperature

To determine the most efficient temperature for optimum extraction of mesotrione from the four soils, three temperatures (50°C, 100°C, and 150°C) were

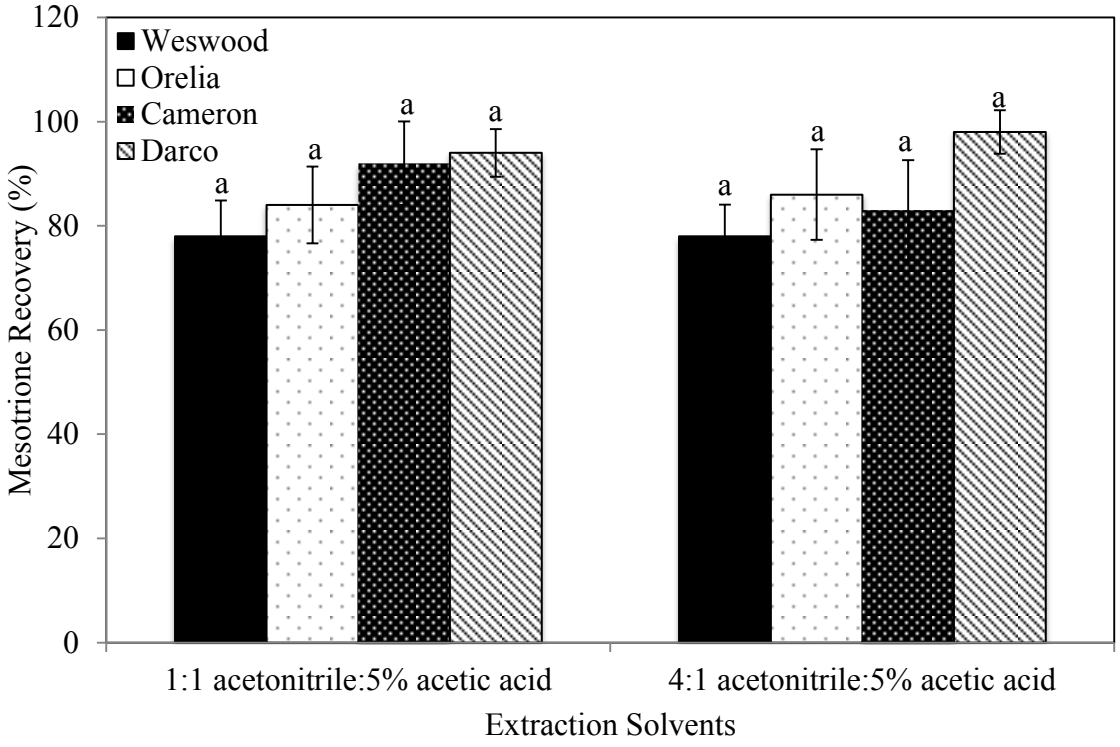

Fig. 2 Mesotrione recovery from four soils using two extraction solvents, 1:1 acetonitrile:5% acetic acid and 4:1 acetonitrile:5% acetic acid. Two static cycles and a temperature of 50°C were used. Error bars represent standard error of the mean. Different letters denote significant differences ($P \leq 0.05$) within each extraction solvent group. The figure represents statistical differences between the four soils for each extraction solvent.

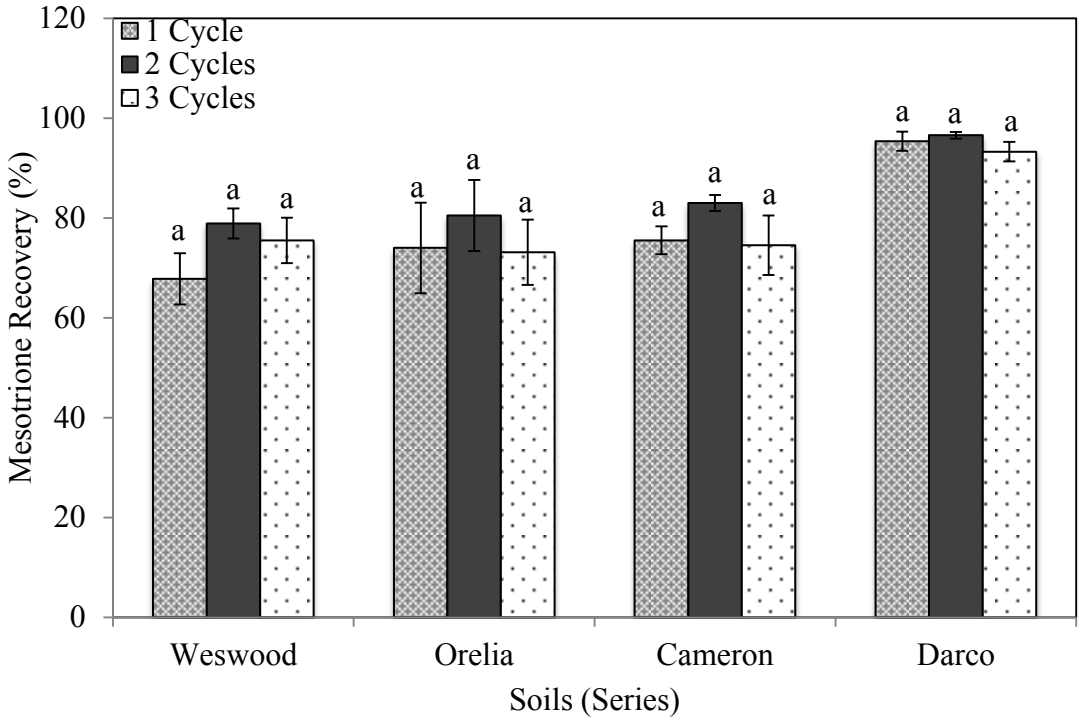

Fig. 3 Influence of 1, 2, and 3 extraction cycles on mesotrione recovery from four soils. A 4:1 acetonitrile:5% acetic acid solvent and temperature of 50°C were used. Error bars represent standard error of the mean. Different alphabet letters denote significant differences ($P \leq 0.05$). The figure represents statistical differences between the three extraction cycles for each soil.

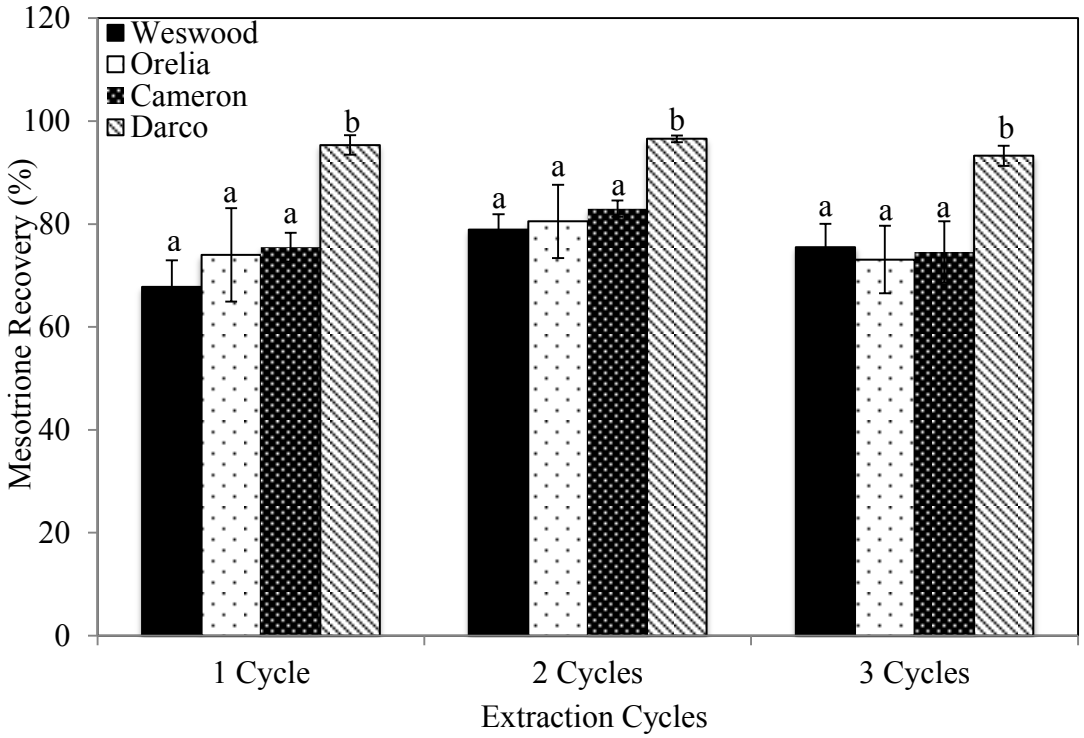

Fig. 4 Mesotrione recovery from four soils using three cycles (1, 2, and 3). A 4:1 acetonitrile:5% acetic acid solvent and temperature of 50°C were used. Error bars represent standard error of the mean. Different letters denote significant differences ($P \leq 0.05$) within each extraction cycle group. The figure represents statistical differences between the four soils for each extraction cycle.

evaluated using a 4:1, acetonitrile:5% acetic acid extraction solvent and 2 static cycles. The results indicate statistically significant differences for the temperature of 150°C (Fig. 5), resulting in the lowest mesotrione recoveries. Mesotrione begins to slowly decompose at temperatures close to its melting point of 165°C (Senseman et al. 2007). Perhaps, using a temperature (150°C) close to the melting point (165°C) in combination with high pressure resulted in a slight decomposition of the compound, which could explain the lower mesotrione recoveries observed for this extraction temperature. Furthermore, following extraction the extracted solvent appeared somewhat cloudy for this temperature, with the most cloudiness being observed in the Darco soil which gave the lowest recoveries. It is possible that the high temperature caused the extraction of other substances found in the soil matrix. Although there were no significant differences observed for temperatures of 50°C and 100°C, the temperature of 50°C was found to be efficient for optimum extraction of mesotrione, demonstrating higher recoveries in the four soils. Mesotrione recoveries did not show significant extraction differences in any three temperatures for the four soils (Fig. 6).

Method validation

In order to evaluate the accuracy of the method, a validation test was carried out by fortifying samples with a range of mesotrione concentrations consisting of 8, 10, 12, 14, and 16 $\mu g\ g^{-1}$. Samples were extracted with those parameters that were previously identified as optimal, a 4:1, acetonitrile:5% acetic acid extraction solvent at 50°C with two static cycles.

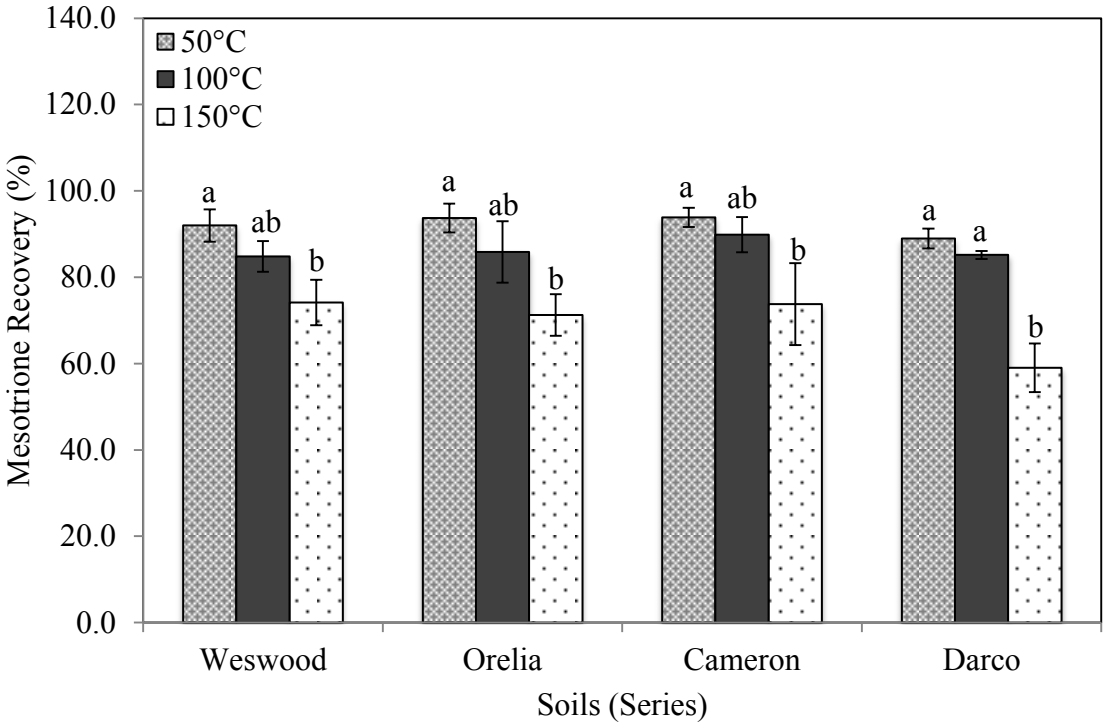

Fig. 5 Influence of temperatures 50˚C, 100˚C, and 150˚C, on mesotrione recovery from four soils. A 4:1 acetonitrile:5% acetic acid solvent and two static cycles were used. Error bars represent standard error of the mean. Different letters denote significant differences ($P \leq 0.05$). The figure represents statistical differences between the three temperatures for each soil.

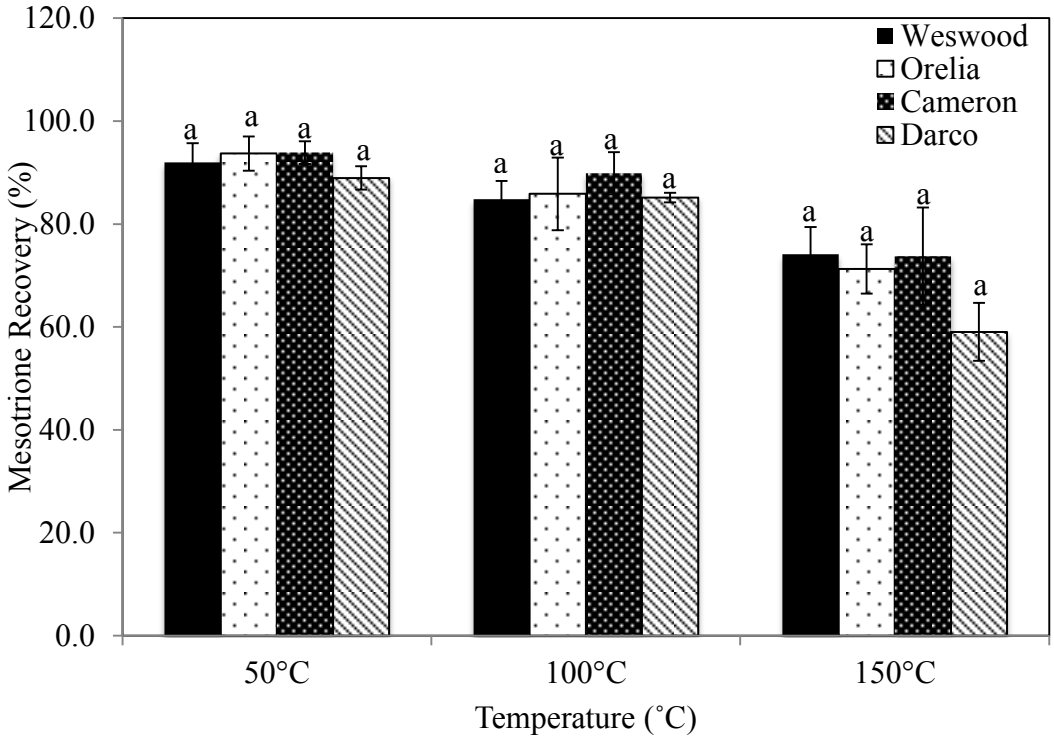

Fig. 6 Mesotrione recovery from four soils using three temperatures (50˚C, 100˚C, and 150˚C). A 4:1 acetonitrile:5% acetic acid solvent and two static cycles were used. Error bars represent standard error of the mean. Different letters denote significant differences ($P \leq 0.05$) within each extraction temperature group. The figure represents statistical differences between the four soils for each temperature.

The mean recoveries were 85 to 86% for the Weswood, 76 to 87% for the Orelia, 85 to 94% for the Cameron, and 79 to 84% for the Darco for the range of mesotrione concentrations used in the recovery test. The relative standard deviation (RSD) determines the accuracy of the method. The % RSD's were 0.5 to 2.5% for the Weswood, 0.8 to 2.6% for the Orelia, 1.0 to 2.8% for the Cameron, and 1.5 to 2.6% for the Darco (Table 3), Higher RSD means that the values being evaluated are widely spread from its average. The lower RSDs obtained indicate an acceptable accuracy for the method.

For the instrumental precision, intra-day (on the same day) and inter-day (three different days) precision was determined. The intra-day precision RSD was 0.8% (Table 4) and the inter-day precision RSD ranged from 0.3 to 0.9% for the retention times and 0.5 to 5% for the peak areas (Table 5).

The specificity of the method was assessed. The diode-array detector was used to acquire spectral data. The spectral data was used to compare the spectra of the standard and the sample to unequivocally assess the presence of mesotrione. No interfering peaks for the determination of mesotrione were observed.

The calibration curve for the linearity between five concentrations of mesotrione and the corresponding peak areas were constructed. Linear ranges showed good correlation ($R^2 > 0.99$) with the concentrations used (Fig. 7).

The LOD and LOQ were established for sensitivity. LOD and LOQ were determined separately for each soil because differences due to organic components of the soil were expected. The LOD ranged from 0.3 μg g $soil^{-1}$ to 0.5 μg g $soil^{-1}$ and the

Table 3 Mesotrione recovery study for four soils at multiple fortified concentrations.

Soil	Standard Added (μg g^{-1})	Recovery (%)	RSD %
Weswood	8	85	1.8
	10	86	2.5
	12	85	1.6
	14	85	1.3
	16	85	0.5
Orelia	8	81	1.6
	10	81	2.6
	12	87	1.1
	14	76	0.8
	16	81	2.4
Cameron	8	85	1.0
	10	93	2.6
	12	91	1.6
	14	94	2.8
	16	92	1.5
Darco	8	84	2.4
	10	83	1.5
	12	79	2.4
	14	81	2.6
	16	82	1.6

Table 4 Summary of intra-day area response data for mesotrione at a 0.5 μg g^{-1} concentration.

Injection	Area Response
1	498719
2	491643
3	493597
4	501103
5	500907
6	502653
Mean	498104
RSD[a] (%)	0.8

[a]Relative Standard Deviation (RSD). Higher RSD means that the values being evaluated are widely spread from its average. The lower RSDs obtained indicate an acceptable accuracy for the method.

Table 5 Summary of inter-day data for mesotrione at 1, 3, and 5 μg ml^{-1} concentrations.

Concentrations	Inter-day					
	Day 1		Day 2		Day 3	
	Rt[a]	PA[b]	Rt	PA	Rt	PA
1	0.3	5.0	0.7	3.5	0.8	1.7
3	0.9	0.5	0.9	1.4	0.6	2.4
5	0.8	1.8	0.8	1.2	0.8	1.4

[a]relative standard deviation (%) of retention time (Rt).
[b]relative standard deviation (%) peak area (PA).

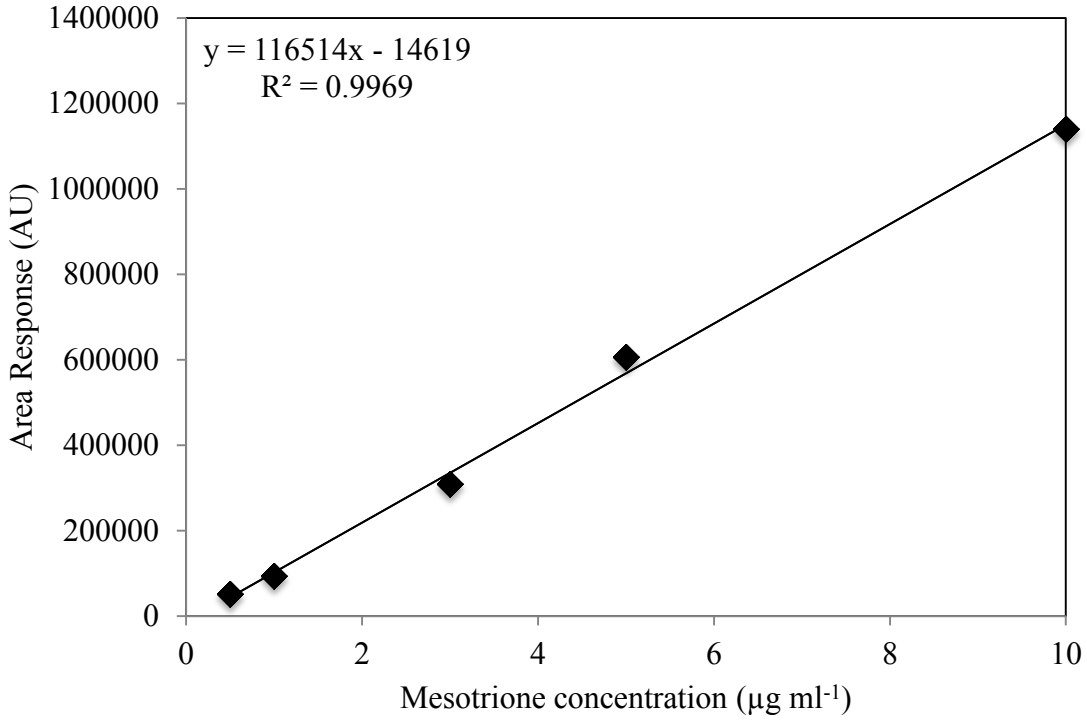

Fig. 7 Mesotrione linearity plot analyzed HPLC for concentrations ranging from 0.5 to 10 µg ml^{-1}.

Table 6 Summary of LOD and LOQ for mesotrione in four soils (μg g soil^{-1})**.**

Soil	LOD[a]	LOQ[b]
Weswood	0.3	1.2
Orelia	0.3	1.1
Cameron	0.4	0.8
Darco	0.5	1.2

[a]Limit of Detection (LOD).
[b]Limit of Quantitation (LOQ).

LOQ ranged from 0.8 μg g soil^{-1} to 1.2 μg g soil^{-1}, respectively, for mesotrione (Table 6).

Solid-phase extraction has been used to extract mesotrione from soil samples, resulting in mesotrione recoveries of 96% (Barchanska et al. 2012). The present study reports the efficient conditions to extract mesotrione in four soils, resulting in acceptable recoveries in the method validation recovery study, ranging from 76 to 94%.

This technique allows for improved sample analysis by reducing extraction time to 23 minutes per sample with ASE as compared to 4 to 48 hours per sample with Soxhlet or 30 min to 1 hr using microwave assisted solvent extraction (Giergielewicz-Mozajska et al. 2001). These findings clearly indicate that ASE can be used successfully to extract mesotrione from soils having a wide range of chemical and physical properties and ultimately further study mesotrione degradation and persistence in soil.

CHAPTER III

IMPACT OF COMBINED ATRAZINE APPLICATION ON MESOTRIONE DEGRADATION IN SOIL

Introduction

Mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), is a pre- and post-emergence herbicide used to control broadleaf weeds in corn (Mitchell et al. 2001). This herbicide is marketed by Syngenta Crop Protection AG, under the commercial formulation Callisto®. Mesotrione has been used as a replacement for the herbicide atrazine (6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine), a widely used pre- and post-emergence herbicide that has been banned in several European countries including France, Italy, Germany, and Sweden (Durand et al. 2006; Swanton et al. 2007; Crouzet et al. 2010; Crouzet et al. 2013). The banning of atrazine was a result of atrazine's persistence in the environment, contamination of surface and groundwater sources, and potential negative human health impacts. In the U.S., mesotrione is being used alone and in combination with atrazine, to control several problematic weed species (Armel et al. 2003; Creech et al. 2004; Armel et al. 2005). Studies have shown that when two herbicides are used in mixtures, the herbicides often persist longer in the environment (Krutz et al. 2003; Li et al. 2008; Tejada 2009), increasing the chances of environmental contamination. Although studies have evaluated mesotrione degradation in soil when applied alone (Dyson et al. 2002; Chaabane et al. 2008), it is necessary to study the herbicide mixtures currently used in fields in order to assess the true impact they can have on chemical persistence in the environment. No

studies were found in the literature that evaluated the potential effect that mixtures of mesotrione and atrazine could have on chemical degradation. The objective of this paper was to study the effect of atrazine on mesotrione degradation in soil.

Materials and methods

Soil collection and characterization

The soils used in this study included a Cameron silty clay (clayey over loamy, mixed, active, hyperthermic Vertic Haplustolls), Darco loamy sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults), and Orelia sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Argiustrolls). The soils were analyzed by Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas. The soils were collected from the top horizon of the soil (15-cm upper layer), brought to the laboratory, air-dried, and then passed through a 2-mm sieve for removal of large particles and non-decomposed plant residues. The soils were stored at room temperature (24°C ± 2°C) prior to the studies. Preliminary quality control assurance indicated no detectable mesotrione or atrazine in the soil samples (data not shown).

Chemicals

Analytical standards of atrazine and mesotrione (98.8% and 99.9% purity, respectively; Sigma-Aldrich, St. Louis, MO) were used to prepare stock solutions in HPLC grade acetonitrile. Stock solutions of mesotrione and atrazine were applied to 30 g of soil in 3 ml of acetonitrile at a concentration of 100 μg ml^{-1} (10 μg g^{-1}) and 1000 μg ml^{-1} (100 μg g^{-1}), respectively. The herbicide rates used in this study were approximately

33 times higher than the maximum recommended amount to be applied in a cropping season (mesotrione, 0.225 kg ha^{-1}; atrazine 2.2 kg ha^{-1}). The herbicide rates were based on recommended application rates and adjusted by an effective interaction depth of 5 cm for both mesotrione and atrazine. Mobility studies of mesotrione in soil have demonstrated that the greatest concentration of mesotrione remained in the upper 2 to 4 cm of soil 2.5 months after its application (Rouchaud et al. 2001). The effective interaction depth can be described as the depth in the soil profile where the herbicide is likely to be found following field application and proper activation. This adjustment permits for a more realistic concentration estimate of the herbicide in the soil.

Mesotrione degradation in soil

Air-dried samples of soil (30 g) were placed in glass jars and were rewetted (20% w/w, dry weight basis) to re-establish microbial activity. The glass jars were pre-incubated at 28°C in the dark for 10 days prior to herbicide application. The moisture content was maintained by adding distilled water twice a week. Herbicides were added to soil samples in 3 ml of acetonitrile. Soils were then incubated at 28°C in the dark and 10 g sub-samples were collected at 0 (5 hours after application), 3, 8, 14, 30, and 60 days after the experiment began and frozen at -80°C in sterile plastic Whirl-Pak® bags until analysis.

Extraction procedure

An accelerated solvent extraction (ASE) method was developed in previous experiments to extract mesotrione from soil. Three samples at a time were removed from the freezer and transferred into 50-ml glass beakers. Removing the soil samples from the

Whirl-Pak® bags to the glass beakers in a frozen state minimized soil and chemical loss during sample handling. The samples were defrosted for approximately 15 min. After the samples were defrosted, 2 g of Hydromatrix® (diatomaceous earth, VWR, Radnor, PA) was added. Samples mixed with Hydromatrix® (VWR, Radnor, PA), were then transferred into the 22-ml ASE extraction cells assembled with a 19-mm glass fiber filter (Dionex Corp., Sunnyvale, CA), at the bottom of the cell. The Hydromatrix® allowed for the absorption of moisture and facilitated the transfer of the soil sample into the ASE extraction cells. The samples were then loaded onto the ASE system where they were preheated for 2 min before being filled up with the extraction solvent consisting of a 4:1 acetonitrile:5% acetic acid. The samples were then heated to 50°C and pressurized to 10.3 MPa for 5 min to achieve thermal equilibrium. Static cycles (2 cycles) were then initiated, maintaining pressure and temperature for 5 min. The cells were partially flushed (60% of the cell volume) and fresh solvent filled the cell at the end of each cycle. The final step consisted of a purging event of 2 min, where a stream of nitrogen gas discharged the aqueous sample into the collection vials.

Sample preparation

The volume of aqueous sample discharged into the collection vials was measured using a 100-ml graduated cylinder. All samples were brought up to a 40-ml volume using the extraction solvent. A 1-ml aliquot of the final volume was removed and filtered through 0.45-µm Acrodisc® GHP syringe filters (Waters Corp., Milford, MA) using disposable plastic 3-ml syringes with a Luer-Lok™ tip (BD, Franklin Lakes, NJ) into 1-ml clear glass shell vials with polyethylene snap caps (Waters Corp., Milford, MA).

HPLC analysis

Extractions from soil were analyzed using a Waters HPLC system consisting of a Model 616 pump, Model 717 autosampler, Model 600S controller, and a Model 996 photodiode array detector (Waters Corp., Milford, MA). A Symmetry® Shield RP8, 3.5-mm, C8, 2.1 x 150-mm column (Waters Corp., Milford, MA) was used for chemical separation analysis. An isocratic mobile phase was prepared consisting of 35% acetonitrile, 64.5% deionized water and 0.5% formic acid. The mobile phase was filtered using 0.45-µm Millipore™ Durapore® membrane filters (EMD Millipore Corp., Billerica, MA) and was degassed before running through the HPLC system. Samples were analyzed for 18 min using a flow rate of 0.2 ml min^{-1}. The sample injection volume was 10 µL. The retention time for mesotrione was 13.1 ± 0.4 min and for atrazine it was 16.0 ± 0.4 min. Samples were analyzed at 270 nm for mesotrione (Halle et al. 2010) and 230 nm for atrazine (Pinto et al. 2000). Calibration standards were prepared in acetonitrile at 0.5, 1, 3, 5, and 10 µg ml^{-1}. These concentrations encompassed the expected range of responses in the final sample aliquots after extraction and concentration. The R^2 for the calibration curves prepared during the study were above 0.990. Calibration standards were included in every analyzed sample set.

Data analysis

Numerous studies have applied a first-order model to describe the degradation of herbicides in soil (Nicholls et al. 1982; Jenks et al. 1998; Lancaster et al. 2008; Camargo et al. 2013). Degradation rate constants were estimated by linear regression from transformed first-order rate equation, equation 1.

$$ln\frac{C}{Co} = -kt \quad (1)$$

where C_o is the initial herbicide concentration, *k* is the rate constant, and C is the concentration of the herbicide at time *t*. Here a plot of ln (C/C_o) versus time is constructed, resulting in a straight line where the slope is equal to –*k* at a constant temperature. The half-life coefficient, $t_{½}$ (days) was calculated using equation 2 described in other studies (Martins and Mermoud 1998).

$$t_{1/2} = \frac{0.693}{k} \quad (2)$$

where *k* is the first-order degradation constant and $t_{1/2}$ is the half-life. This study was conducted as a completely randomized design consisting of three replicates per treatment and the experiment being repeated twice. Variances were tested for homogeneity using the Levene's test and the comparison of group means within each incubation day were analyzed using Fisher's Least Significant Difference (LSD) test at the 5% level of significance (Benedetti et al. 1997) using Statistical Analysis Systems Version 9.3 (SAS Institute, Inc., Cary, NC).

Results and discussion

Selected characteristics of the three soils used in this study are presented in Table 7. The Cameron, Darco, and Orelia soils used in this study were identified as having a texture class of clay, loamy sand, and sandy clay loam, respectively. The Cameron soil has the highest percent clay (42%), organic matter (1.94%), and pH (8.1) followed by the Orelia soil (34% clay, 1.58% organic matter, and 7.9 pH) and lastly, the Darco soil (5% clay, 1.20% organic matter, and 6.0 pH). Preliminary studies were conducted to

determine if mesotrione or atrazine was present in the collected soil samples. No mesotrione or atrazine residues were detected in untreated soil samples (data not shown).

The Levene's test of homogeneity of variances was performed on the herbicide concentrations recovered at each sampling day for both experimental runs and the three soils. This test was done to determine if the data from the repeated experiments could be pooled. Variances were determined to be homogeneous according to the Levene's test. Table 8 presents the p-values from the Levene's test for mesotrione when applied alone and in the mesotrione + atrazine treatments. All the p-values obtained were > 0.05, suggesting that the variances for the two experimental runs were homogeneous. Table 9 presents the p-values from the Levene's test for atrazine in the mesotrione + atrazine treatments. Again, all the p-values obtained were > 0.05, indicating homogeneity of variances from the two experimental runs. As a result of having homogeneous variances, the data from the two experimental runs was combined for further analysis.

This study focused on evaluating the unknown effects of atrazine and mesotrione mixtures on soil degradation. A Fisher's LSD test at the 5% level of significance was conducted. Table 10 presents the treatment means ($\mu g\ g^{-1}$) and statistical differences observed for mesotrione when applied alone and in the mesotrione + atrazine treatments. The results show that the extractable mesotrione at day 60 in the Cameron soil declined to 55 (4.8 $\mu g\ g\ soil^{-1}$) and 74% (6.7 $\mu g\ g\ soil^{-1}$) in the mesotrione and mesotrione + atrazine treatments, respectively. The concentration of mesotrione also decreased more rapidly when applied alone in this soil, with statistical differences among treatments being observed at days 30 and 60 (Table 10, Fig. 8).

Table 7 Selected characteristics of soils used in this study[a].

Parameters	Soil characterization		
Soils collected	Weslaco, TX	Overton, TX	Corpus Christi, TX
Soil series name	Cameron	Darco	Orelia
Texture class[b]	C	LS	SCL
Sand, %	43	88	50
Silt, %	15	7	16
Clay, %	42	5	34
Organic matter, %	1.94	1.20	1.58
pH	8.1	6.0	7.9

[a]Samples were analyzed by the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas.
[b]C, clay; LS, loamy sand; SCL, sandy clay loam.

Table 8 P-values from Levene's test for homogeneity of variances for mesotrione when applied alone and in combination with atrazine.

Soil series	Day	Treatment p-values	
		Mesotrione	Mesotrione + atrazine
Cameron	0	1.00	0.71
	3	0.16	0.21
	8	0.13	0.17
	14	0.19	0.25
	30	0.76	0.49
	60	0.27	1.00
Darco	0	1.00	0.71
	3	0.61	0.22
	8	0.51	0.75
	14	0.14	0.26
	30	0.22	0.14
	60	0.83	0.18
Orelia	0	1.00	0.71
	3	0.71	0.11
	8	0.13	0.15
	14	0.26	0.22
	30	0.28	0.16
	60	0.57	0.12

Table 9 P-values from Levene's test for homogeneity of variance for atrazine when applied in combination with mesotrione.

Day	Treatment p-values (Mesotrione + atrazine)		
	Cameron	Darco	Orelia
0	0.16	0.12	0.13
3	0.16	0.36	0.22
8	0.19	0.13	0.13
14	0.67	0.39	0.99
30	0.96	0.27	0.14
60	0.14	0.17	0.13

The Darco soil showed that the extractable mesotrione at day 60 declined to 40 (4 μg g soil^{-1}) and 59% (5.4 μg g soil^{-1}) in the mesotrione and mesotrione + atrazine treatments, respectively. The concentration of mesotrione decreased more rapidly in this soil when applied alone, with statistical differences among treatments being observed only at day 60 (Table 10, Fig. 9). The Orelia soil showed that the extractable mesotrione at day 60 declined to 74 (7.2 μg g soil^{-1}) and 81% (7.6 μg g soil^{-1}) in the mesotrione and mesotrione + atrazine treatments, respectively. There was no statistical difference among treatments throughout the incubation period in the Orelia soil (Table 10, Fig. 10).

The results show that the Darco soil exhibited the greatest mesotrione degradation, followed by the Cameron soil. The data obtained from the Orelia soil suggests that minimal mesotrione degradation occurred through the duration of the study in that soil. Furthermore, a significant decline in atrazine can be observed following day 30 in the Cameron and Darco but not the Orelia soil (Fig. 11). A Fisher's LSD test at the 5% level of significance confirmed the statistical differences observed between the three soils. Table 11 presents the treatment means (μg g^{-1}) and statistical differences observed for atrazine when applied in combination with mesotrione. Statistical differences among the soils were observed at day 60 (Table 11, Fig. 11).

Since the degradation of mesotrione was slowest in the Orelia soil, it is possible that the soil microbial populations present in this soil may have continued to degrade the native organic matter in the soil, and were unaffected by the addition of the relative amounts of added herbicides.

Table 10 Treatment means and statistical differences for mesotrione when applied alone and in combination with atrazine.

Soil	Day	Treatment means($\mu g\ g^{-1}$)	
		Mesotrione	Mesotrione + atrazine
Cameron	0	8.65[a]	9.03[a]
	3	7.25[a]	8.33[a]
	8	7.00[a]	6.92[a]
	14	6.92[a]	7.05[a]
	30	6.90[a]	8.14[b]
	60	4.85[a]	6.68[b]
Darco	0	10.00[a]	9.17[a]
	3	9.47[a]	8.85[a]
	8	6.90[a]	8.03[a]
	14	5.97[a]	5.85[a]
	30	6.13[a]	5.48[a]
	60	3.97[a]	5.40[b]
Orelia	0	9.75[a]	9.27[a]
	3	8.78[a]	8.92[a]
	8	7.92[a]	8.67[a]
	14	7.13[a]	7.37[a]
	30	7.91[a]	7.45[a]
	60	7.17[a]	7.62[a]

Means followed by different letters in a row indicate significant differences of mesotrione treatments when applied alone and in combination with atrazine ($p < 0.05$).

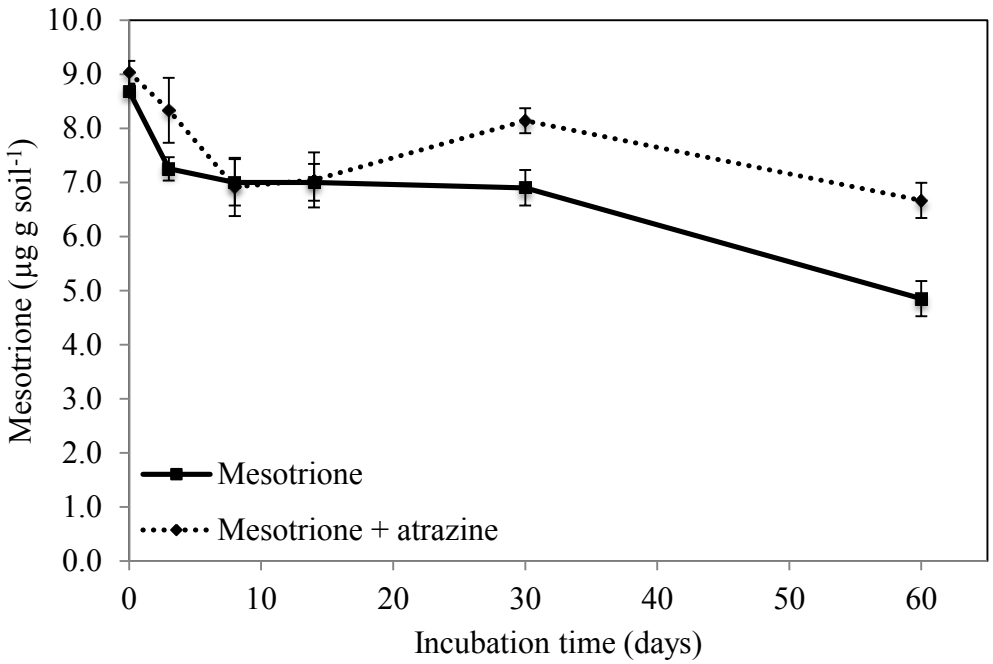

Fig. 8 Mesotrione (■) and mesotrione + atrazine (♦) degradation trend as affected by atrazine for the Cameron soil series during the 60 days of incubation. Error bars represent the standard error of the mean.

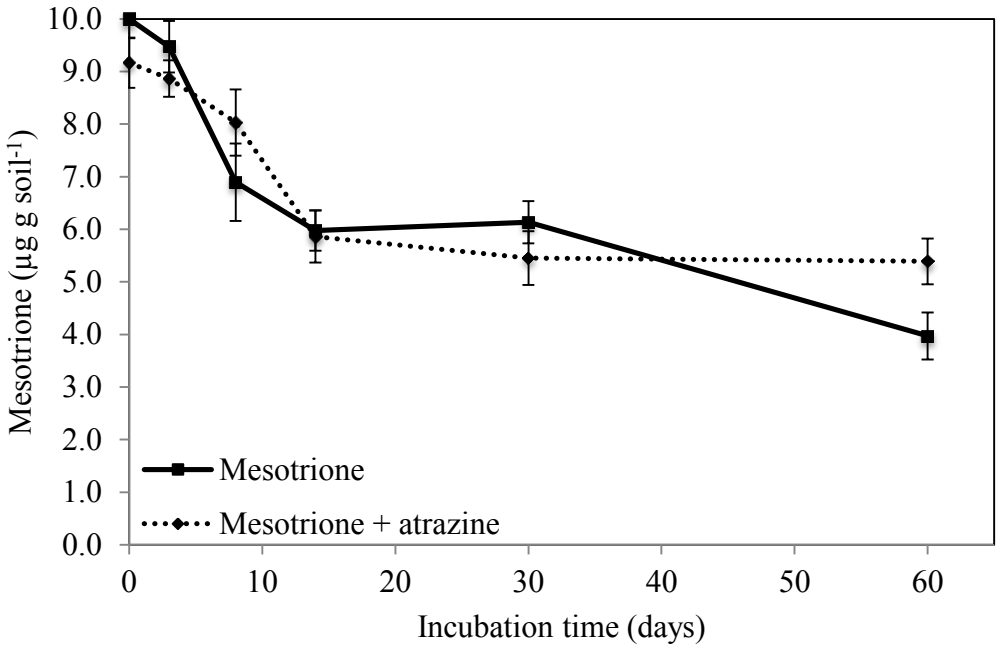

Fig. 9 Mesotrione (■) and mesotrione + atrazine (♦) degradation trend as affected by atrazine for the Darco soil series during the 60 days of incubation. Error bars represent the standard error of the mean.

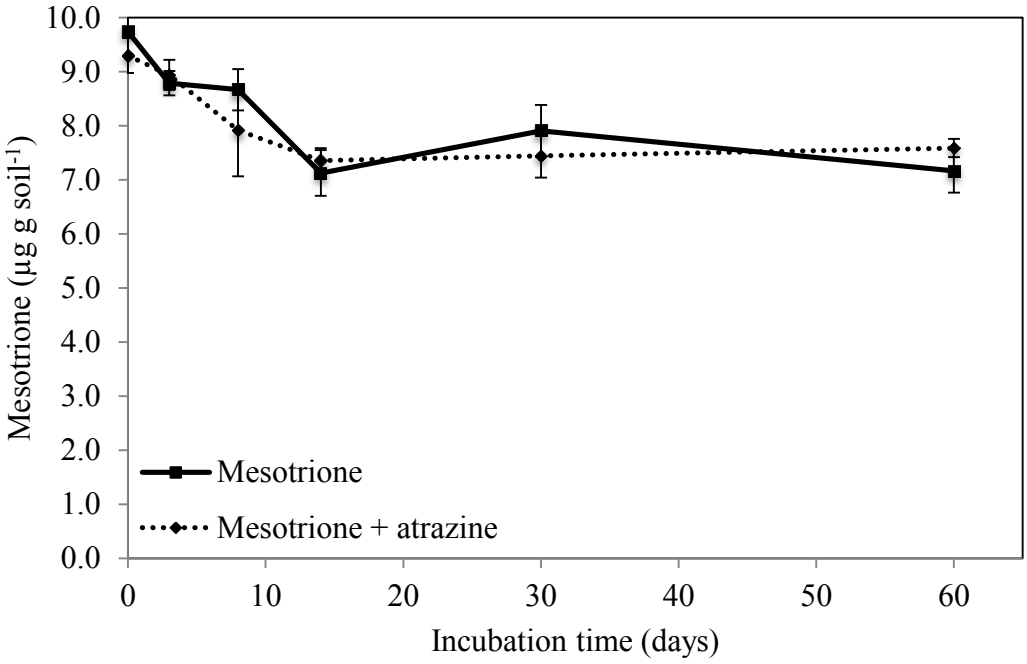

Fig. 10 Mesotrione (■) and mesotrione + atrazine (♦) degradation trend as affected by atrazine for the Orelia soil series during the 60 days of incubation. Error bars represent the standard error of the mean.

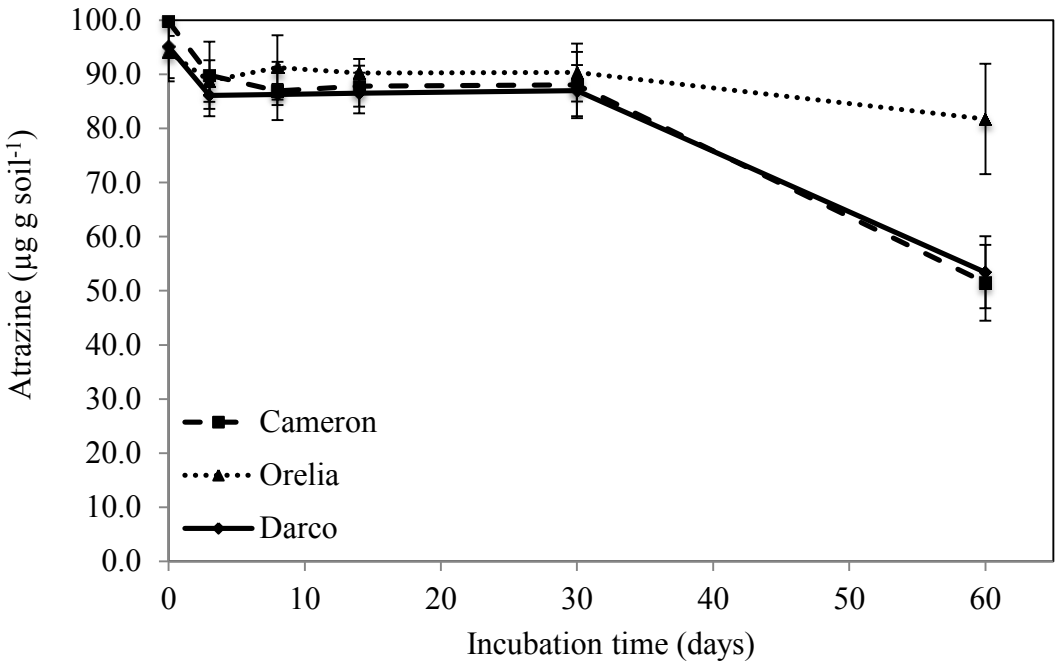

Fig. 11 Atrazine degradation trend for the Cameron (■), Darco (♦), and Orelia (▲) soil series during the 60 days of incubation. Error bars represent the standard error of the mean.

Table 11 Treatment means and statistical differences for atrazine when applied in combination with mesotrione.

Day	Treatment means (μg g^{-1}) (Mesotrione + atrazine)		
	Cameron	Darco	Orelia
0	99.78[a]	95.10[a]	94.15[a]
3	89.80[a]	86.13 [a]	88.73[a]
8	86.92 [a]	86.27[a]	91.23[a]
14	87.78 [a]	86.52[a]	90.25[a]
30	88.03 [a]	86.97[a]	90.33[a]
60	51.48 [a]	53.40[a]	83.08[b]

Means followed by different letters in a row indicate significant differences of atrazine in mesotrione + atrazine treatments between the three soils ($p < 0.05$).

It is also possible that the slow degradation of the compounds in this soil through the duration of the study could have occurred because the soil microbial populations present did not possess the enzyme systems necessary to degrade the compounds or some other factor caused their activity level to be lower than that found in the Cameron and Darco soils.

Furthermore, the availability of the herbicides could have been impacted by the extent of adsorption and desorption of mesotrione in soil. There was not a clear distinction in the Orelia soil properties when compared to the Cameron and Darco soil, being the in the middle in terms of percent sand, clay, organic matter, and pH. A study conducted by Dyson et al. (2002), looked at the adsorption and degradation of mesotrione in 15 different soils from Europe and the U.S. to understand the influence of soil textures, soil pH, and organic carbon content. When the soil pH rises, the anionic form of mesotrione would predominate, reducing the adsorption of mesotrione to the soil surfaces (Dyson et al. 2002). The soil pH in the Orelia soil is 7.9 and you would expect less adsorption of mesotrione to soil, making it more available for soil microbes to degrade but we see the opposite occur in this soil. Dyson et al. (2002), also found that organic matter can account for some of the adsorption not accounted for by soil pH. Even though it is expected for the Orelia soil to experience a decrease in mesotrione adsorption, the chemical compounds could be coming into contact with the organic matter found in that soil, making it less available for the specific microbial population(s) in that soil to degrade the herbicide. Adsorption to organic matter could be taking place

in the Cameron and Darco soil but at a lower extent than the Orelia soil, causing a higher rate of degradation in those soils.

Another possibility is that the soil microbial populations present in the three soils could have had a different susceptibility to the mesotrione + atrazine treatment. It is possible that the mesotrione + atrazine treatment in the Cameron and Darco soils could have had a toxic effect to some of the most active degraders possessing the enzyme systems necessary to degrade the compounds. This toxicity could not have been immediate but after continued exposure to the herbicide mixture, for statistical differences among the mesotrione and mesotrione + atrazine treatments were not observed until after 30 days and 60 days in the Cameron and Darco soils, respectively. On the other hand, the soil microbial populations in the Orelia soil could have been more susceptible to not only the mesotrione + atrazine treatment but also the mesotrione treatment, causing an immediate toxic effect, reducing the degradation rate in both treatments, resulting in no statistical differences among treatments through the duration of the study.

There have not been many studies on the toxicity of mesotrione on specific microbial groups, but Bonnet et al. 2008 looked at the toxicity of mesotrione and another herbicide, sulcotrione, towards two reference environmental microorganisms: *Tetrahymena pyriformis* and *Vibrio fischeri*. These two microorganisms are frequently used in ecotoxicology to study the effects of toxic chemicals on biological organisms. They found on their toxicity assessment towards *T. pyruformis* and *V. fischeri* that the commercial product, Callisto® was more toxic than the analytical standards but

nonetheless, the analytical standards still caused a toxic effect to these reference microorganisms, indicating the possibility of toxicity towards other soil microorganisms.

This observed trend of atrazine degradation could also explain the significant differences observed between mesotrione and mesotrione + atrazine treatments in the Cameron and Darco soils. As it was previously discussed, the results suggest that the addition of atrazine reduced mesotrione degradation when comparing mesotrione-only treatment with the mesotrione + atrazine mixture. Perhaps there is a greater microbial preference for the substrate atrazine in the Cameron and Darco soils when it is combined with mesotrione. When atrazine is being heavily degraded, some process may be taking place that as a result, a decrease in mesotrione degradation is observed. This trend in atrazine degradation was not seen in the Orelia soil, and might again, be explained by potential differences in microbial populations present and their preferences in substrate degradation.

A first-order kinetic model was used to describe mesotrione degradation in this study. Linear regression graphs of the natural log of concentration remaining/initial concentration and days after application for the three soils were constructed (Figs. 12, 13, and 14). The calculated degradation rate constant, half-life, and coefficient of determination for each line are presented in Table 12. The data obtained shows that the calculated mesotrione half-life increased in the mesotrione + atrazine treatments for all soils, further suggesting that the addition of atrazine is the potential cause of reduced mesotrione degradation, even slightly in the Orelia soil.

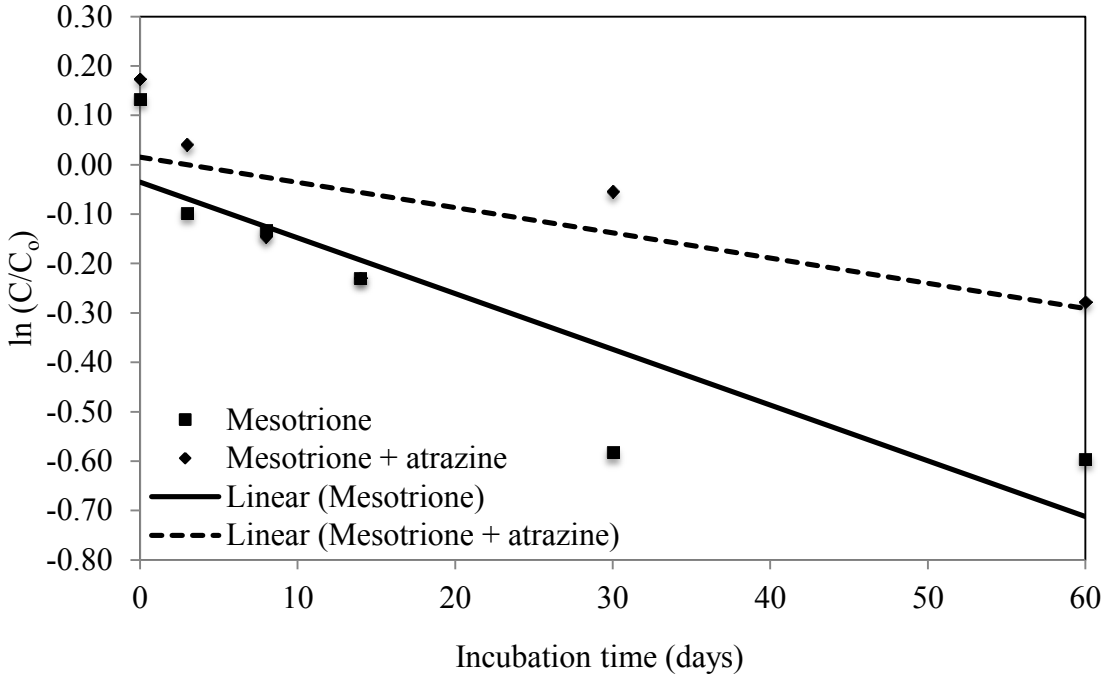

Fig. 12 First-order rate plots for degradation of mesotrione applied alone (■) and with atrazine (♦) for the Cameron soil series. Fitted equations are as follows: y =-0.0113x – 0.0348, mesotione alone; y =-0.0051x + 0.0157, mesotrione + atrazine.

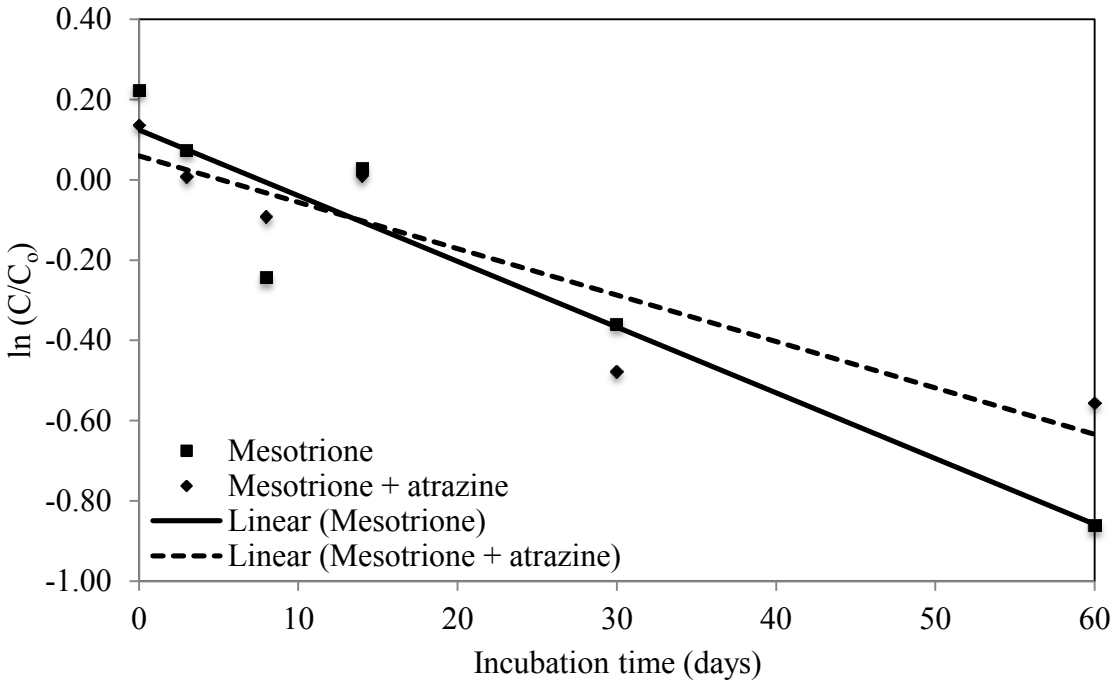

Fig. 13 First-order rate plots for degradation of mesotrione applied alone (■) and with atrazine (♦) for the Darco soil series. Fitted equations are as follows: y =-0.0073x + 0.1691, mesotione alone; y =-0.0061x + 0.1304, mesotrione + atrazine.

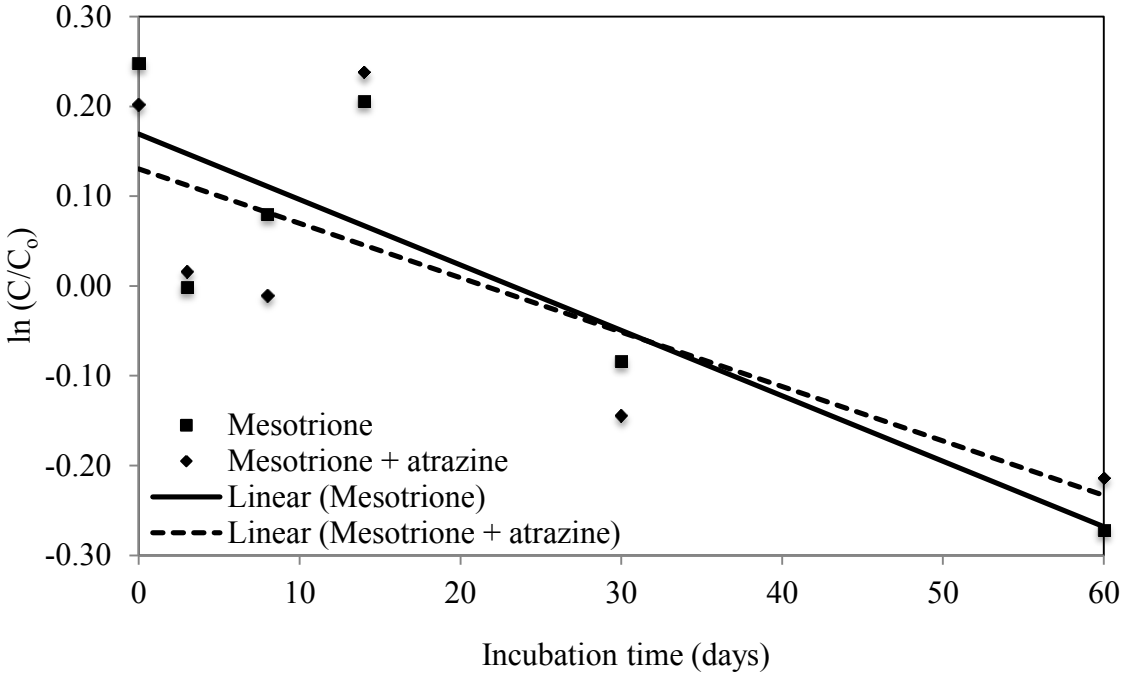

Fig. 14 First-order rate plots for degradation of mesotrione applied alone (■) and with atrazine (♦) for the Orelia soil series. Fitted equations are as follows: y =-0.0164x + 0.1238, mesotione alone; y =-0.0116x + 0.0596, mesotrione + atrazine.

Table 12 First-order rate constant (k), half-life ($t_{1/2}$), and coefficient of determination (R^2) for mesotrione in soils treated with mesotrione alone and in combination with atrazine.

Treatment	Soil series	k	$t_{1/2}$ (days)	R^2
Mesotrione	Cameron	0.0113	61	0.79
	Darco	0.0164	42	0.89
	Orelia	0.0073	95	0.73
Mesotrione + atrazine	Cameron	0.0051	136	0.46
	Darco	0.0116	60	0.84
	Orelia	0.0061	114	0.58

The calculated half-lives for mesotrione alone in the Cameron, Darco, and Orelia soils were 61, 42, and 95 days respectively. Although the obtained half-lives for mesotrione were greater than those reported by Dyson et al. (2002) of 4.5 to 32 days and Chaabane et al. (2008) of 5 to 34 days, the half-life for the Darco soil (42 days) is within the range of reported half-lives for mesotrione of 34 to 50 days (Rouchaud et al. 2001), and the half-life for the Cameron soil is proximal to reported ranges. Even though the half-life of 95 days for the Orelia soil is greater than reported half-lives, this could have been influenced by differences in the physicochemical properties of soils (pH and organic matter content) and/or the biological properties (distribution and activity of microbial populations present). In the mesotrione + atrazine treatments, the calculated half-lives in the Cameron, Darco, and Orelia soils were 136, 60, and 114 days, respectively, further demonstrating that the addition of atrazine reduced mesotrione degradation, potentially increasing environmental persistence.

Suppressed degradation caused by other herbicide mixtures has been reported by several researchers (Krutz et al. 2003; Li et al. 2008; Tejada 2009). However, other researchers have reported the opposite effect, actually found such as the enhanced degradation of atrazine when combined with glyphosate (Haney et al. 2002). These differences have been attributed to differences in the herbicides being used, soil properties, applications rates, and microorganisms degrading the various herbicides. This is the first study to evaluate the mesotrione + atrazine mixture despite its current use to control undesirable vegetation (Armel et al. 2003; Creech et al. 2004; Armel et al. 2005). The results obtained in this study demonstrated that mesotrione + atrazine

herbicide mixtures have the potential to decrease mesotrione degradation in soils. However, it remains unclear whether the reduced degradation is due to the combined impacts of the herbicides, varying soil characteristics, and/or the soil microbial populations present in each soil.

CHAPTER IV

THE EFFECT OF MESOTRIONE AND ATRAZINE ON SOIL MICROBIAL RESPIRATION

Introduction

In agriculture, herbicides are used to reduce and alleviate weed problems that pose a threat to crop yields. The addition of herbicides may change the microbial populations present in soil and their overall activity, by either stimulating or inhibiting microbial respiration. Measurements of soil respiration can be used to observe the impacts that herbicides could have on soil microbial populations.

An increase in soil microbial activity due to the addition of herbicides has been observed. Wardle and Parkinson (1990) determined that the addition of glyphosate increases bacterial numbers and microbial activity in the soil. Similar results have been observed for glyphosate by Haney et al. (2000) and Busse et al. (2001), where soil microbial activity is increased. Studies with different herbicides such as atrazine, have also found that the addition of atrazine increases measurable microbiological parameters in soil (Moreno et al. 2007). The opposite effect on soil microbial activity has also been observed with the addition of herbicides. Accinelli et al (2002) evaluated the short-time effects of six pure and formulated herbicides on soil microbial activity and biomass. They found that application rates at normal agricultural rates did not cause a significant effect on soil microbial activity but when the rates were increased, this led to a significant decrease of soil microbial activity. These results were observed with atrazine, terbuthylazine, rimsulfuron, and primisulfuron-methyl.

Mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), is a pre- and post-emergence herbicide used to control broadleaf weeds in corn (Mitchell et al. 2001). This herbicide was first registered for use in the U.S. in 2001 under the trade name Callisto®. Since its registration, mesotrione has been used alone and in combination with atrazine (Armel et al. 2003; Creech et al. 2004; Armel et al. 2005). Santos et al. (2006) showed that herbicide mixtures (fluazifop-*p*-butyl and fomesafen) can cause negative impacts on microbial respiration. Specifically with mesotrione, Joly et al. (2012) evaluated mixtures of mesotrione + *S*-metolachlor and found that soil microbial respiration was not negatively impacted. Although mixtures of mesotrione + *S*-metolachlor do not affect microbial respiration, the impact of mesotrione + atrazine is not known. The objectives of this study were to determine if mesotrione treatments, the addition of atrazine in the mesotrione + atrazine treatments, and if application rates had an impact on soil microbial activity (respiration).

Materials and methods

Soil collection and characterization

The soils used in this study included a Weswood clay loam (fine-silty, mixed superactive, thermic Udifluventic Haplustepts), Cameron silty clay (clayey over loamy, mixed, active, hyperthermic Vertic Haplustolls), Orelia sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Argiustrolls), and a Darco loamy sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults). The soils were analyzed by Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas. The soils were collected from the top horizon of the soil (15-cm upper

layer), brought to the laboratory, air-dried, and then passed through a 2-mm sieve for removal of large particles and non-decomposed plant residues. The soils were stored at room temperature (24°C ± 2°C) prior to the studies. Preliminary quality control assurance indicated no detectable mesotrione or atrazine in the soil samples (data not shown).

Pressure plate analysis

Three replicates of each soil were used for pressure plate analysis. The samples were placed inside rings positioned over a suction plate. Water was added to the plate and samples were allowed to saturate. The chamber that contained the suction plate was covered and sealed. Negative pressure of (-33 kPa) was applied to estimate the field capacity moisture (Maclean and Yager 1970). After 24 hrs, the samples were removed from the suction plate and weighed to acquire the wet weight. The samples were oven dried at 105˚C for 48 hrs to obtain the dry weight. The weight of the water per gram of soil at field capacity was determined for the four soils.

Soil pre-incubation

Fifty-gram portions of oven-dry equivalent soil of soil were weighed into wide-mouth 1-liter glass jars. The soils were re-wetted to 55% field capacity to allow for microbial activity re-establishment. The samples were incubated at 28˚C for 16 days prior to herbicide addition.

Chemicals

Analytical standards of atrazine and mesotrione (98.8% and 99.9% purity, respectively; Sigma-Aldrich, St. Louis, MO) were used to prepare stock solutions in

HPLC grade acetonitrile. Mesotrione and atrazine stock solutions were prepared and stored at 4°C by dissolving the analytical-grade herbicide in acetonitrile to give a concentration of 500 μg ml^{-1} for both mesotrione and atrazine. The stock solution was brought to room temperature before use. Individual standard working solutions were prepared by diluting the stock solution.

Soil fortification

Ten gram portions of each soil were weighed into aluminum tin cups. For the mesotrione treatments, mesotrione was applied in 1 ml of acetonitrile at field rates of 1X (concentration of 8.4 μg ml^{-1}, 0.14 μg g^{-1}), 2X (concentration of 16.8 μg ml^{-1}, 0.28 μg g^{-1}), 4X (concentration of 33.6 μg ml^{-1}, 0.56 μg g^{-1}), and 10X (concentration of 84 μg ml^{-1}, 1.4 μg g^{-1}). For the mesotrione + atrazine treatment, mesotrione and atrazine were applied in 1 ml of acetonitrile at field rates 1X (mesotrione concentration of 8.4 μg ml^{-1}, 0.14 μg g^{-1}; atrazine concentration of 54 μg ml^{-1}, 0.9 μg g^{-1}), 2X (mesotrione concentration of 16.8 μg ml^{-1}, 0.28 μg g^{-1}; atrazine concentration of 108 μg ml^{-1}, 1.8 μg g^{-1}), 4X (mesotrione concentration of 33.6 μg ml^{-1}, 0.56 μg g^{-1}; atrazine concentration 216 μg ml^{-1}, 3.6 μg g^{-1}), and 10X (mesotrione concentration of 84 μg ml^{-1}, 1.4 μg g^{-1}; atrazine concentration 540 μg ml^{-1}, 9 μg g^{-1}). The samples were mixed and placed under a fume hood for 24 hrs to allow the evaporation of the acetonitrile. After the 24 hrs, the 10 grams of fortified soil were mixed into the glass jars, bringing up the total volume of the sample to 60 g. For pre-incubation, the soil had been re-wetted to 55% field capacity. When the herbicide was added, enough water was added to bring the soil moisture to 60% field capacity.

The herbicide rates used in this study were based on recommended application rates of commercial formulations, Callisto® and Callisto® Xtra (Syngenta Crop Protection AG), and were adjusted by an effective interaction depth of 5 cm. This adjustment permits for a more realistic concentration estimate of the herbicide in the soil. Mobility studies of mesotrione in soil have demonstrated that the greatest concentration of mesotrione remained in the upper 2 to 4 cm of soil 2.5 months after its application (Rouchaud et al. 2001).

Soil respiration

A plastic cup containing 15 ml of NaOH was placed in each jar of the mixed samples containing the 60 g of soil to trap evolved CO_2. A 22-ml glass ASE vial containing 20 ml of water was added to each jar to maintain humidity throughout the duration of the study. Soils were incubated at 28˚C and NaOH traps were replaced at 0, 4, 8, 16, 24, 32, 42, and 55 days after the experiment began. The NaOH traps were titrated using 0.5 N HCl in the presence of 1 ml of 50% $BaCl_2$ and 2 drops of phenolphthalein indicator (Anderson, 1982).

Statistical analyses

This study was conducted as a completely randomized design consisting of 3 replicates per treatment and the experiment being repeated twice. Variances were tested for homogeneity using the Levene's test for each sampling day and the comparison of group means were analyzed using Fisher's Least Significant Difference (LSD) test at the 5% level of significance (Benedetti et al. 1997) using Statistical Analysis Systems Version 9.3 (SAS Institute, Inc., Cary, NC).

Results and discussion

Soil characteristics

Soil characteristics of the four soils used in this study are presented in Table 13. The Cameron, Orelia, Weswood, and Darco soils used were identified as having a texture class of clay, sandy clay loam, sandy loam, and loamy sand, respectively. The Cameron soil has the highest percent clay (42%) and organic matter (1.94%). The Orelia soil had the second highest percent clay (34%) and organic matter (1.58%). The Weswood soil had the third highest percent clay (18%) but the lowest organic matter (0.85%) while the Darco soil had the lowest percent clay (5%) but more organic matter (1.20%) than the Weswood soil. The Cameron and Weswood soils had the highest pH (8.1) followed by the Orelia (7.9) and Darco (6) soils. Preliminary studies were conducted to determine if mesotrione was present in the collected soil samples. No mesotrione residues were detected in untreated soil samples (data not shown).

The Levene's test of homogeneity of variances was performed on the mg CO_2-C g soil^{-1} at each sampling day, treatment, four soils, and for both experimental runs. This test was done to determine if the data from the repeated experiments could be pooled. Variances were determined to be homogeneous according to the Levene's test. Table 14 presents the p-values from the Levene's test for the control and mesotrione when applied alone and in the mesotrione + atrazine treatments. All the p-values obtained were > 0.05,

Table 13 Selected characteristics of soils used in this study[a].

Parameters	Soil characterization			
Soils collected	Weslaco, TX	Corpus Christi, TX	College Station, TX	Overton, TX
Soil series name	Cameron	Orelia	Weswood	Darco
Texture class[b]	C	SCL	SL	LS
Sand, %	43	50	25	88
Silt, %	15	16	57	7
Clay, %	42	34	18	5
Organic matter, %	1.94	1.58	0.85	1.20
pH	8.1	7.9	8.1	6.0

[a]Samples were analyzed by the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory, College Station, Texas.
[b]C, clay; SCL, sandy clay loam; SL, silt loam; LS, loamy sand.

Table 14 P-values from Levene's test for homogeneity of variances for the control and mesotrione when applied alone and in combination with atrazine for the each sampling day, rate, and soil.

Soils	Rate	Treatment p-values		
		Mesotrione	Mesotrione + atrazine	Control
Day 0				
Cameron	1X Rate	0.16	0.12	0.12
	2x Rate	0.27	0.12	0.71
	4X Rate	0.16	0.12	0.15
	10X Rate	0.12	0.22	1.00
Darco	1X Rate	0.27	0.12	0.19
	2x Rate	0.12	0.22	0.12
	4X Rate	0.12	1.00	1.00
	10X Rate	0.22	0.50	0.16
Orelia	1X Rate	0.16	0.26	0.16
	2x Rate	1.00	0.35	0.14
	4X Rate	1.00	0.22	1.00
	10X Rate	0.16	1.00	1.00
Weswood	1X Rate	0.16	0.27	0.12
	2x Rate	0.27	0.22	1.00
	4X Rate	1.00	0.22	0.15
	10X Rate	0.12	0.22	0.46
Day 4				
Cameron	1X Rate	0.13	0.16	0.71
	2x Rate	0.71	1.00	0.12
	4X Rate	0.13	0.17	0.12
	10X Rate	0.16	0.16	1.00
Darco	1X Rate	0.15	0.14	0.16
	2x Rate	0.71	0.13	0.38
	4X Rate	0.14	0.50	0.35
	10X Rate	1.00	0.27	0.13
Orelia	1X Rate	0.13	0.19	1.00
	2x Rate	0.12	0.29	0.12
	4X Rate	0.12	0.12	0.17
	10X Rate	0.12	0.71	0.12
Weswood	1X Rate	0.14	0.29	0.12
	2x Rate	0.12	0.16	0.22
	4X Rate	0.12	0.14	0.13
	10X Rate	0.30	0.12	0.27

Table 14 Continued

Soils	Rate	Treatment p-values Mesotrione	Mesotrione + atrazine	Control
Day 8				
Cameron	1X Rate	0.22	0.15	0.14
	2x Rate	0.16	0.13	0.18
	4X Rate	0.19	0.17	0.50
	10X Rate	0.36	0.30	0.50
Darco	1X Rate	0.15	0.12	1.00
	2x Rate	0.27	0.27	0.20
	4X Rate	0.23	0.82	0.55
	10X Rate	0.16	0.13	0.13
Orelia	1X Rate	0.12	1.00	0.13
	2x Rate	0.13	0.71	0.27
	4X Rate	0.14	0.82	0.12
	10X Rate	0.22	0.14	1.00
Weswood	1X Rate	0.18	0.42	1.00
	2x Rate	0.13	1.00	0.51
	4X Rate	0.35	0.13	0.13
	10X Rate	0.74	0.13	1.00
Day 16				
Cameron	1X Rate	0.14	0.82	0.13
	2x Rate	0.12	1.00	0.20
	4X Rate	0.78	0.21	0.45
	10X Rate	0.12	0.12	0.22
Darco	1X Rate	0.33	0.13	0.29
	2x Rate	0.21	0.14	0.12
	4X Rate	0.41	0.20	0.27
	10X Rate	0.22	0.17	0.27
Orelia	1X Rate	0.21	0.12	0.14
	2x Rate	0.16	0.13	0.15
	4X Rate	0.12	0.13	1.00
	10X Rate	0.12	0.12	0.12
Weswood	1X Rate	0.18	0.51	0.37
	2x Rate	0.12	0.71	0.13
	4X Rate	0.28	0.14	0.22
	10X Rate	0.16	0.17	0.40

Table 14 Continued

Soils	Rate	Treatment p-values Mesotrione	Mesotrione + atrazine	Control
Day 24				
Cameron	1X Rate	0.21	0.17	0.33
	2x Rate	1.00	0.13	0.13
	4X Rate	0.46	0.29	0.16
	10X Rate	0.13	0.71	1.00
Darco	1X Rate	0.23	0.12	0.12
	2x Rate	0.13	0.12	0.15
	4X Rate	0.34	0.44	0.15
	10X Rate	0.12	0.40	0.77
Orelia	1X Rate	1.00	0.22	0.13
	2x Rate	0.12	0.27	0.12
	4X Rate	0.13	0.37	0.15
	10X Rate	0.13	0.37	0.14
Weswood	1X Rate	0.21	0.52	0.19
	2x Rate	0.37	0.27	0.13
	4X Rate	0.41	0.12	0.13
	10X Rate	0.71	0.27	1.00
Day 32				
Cameron	1X Rate	0.15	0.71	0.51
	2x Rate	0.44	0.46	0.13
	4X Rate	0.12	0.64	1.00
	10X Rate	0.44	1.00	0.16
Darco	1X Rate	0.17	0.16	0.82
	2x Rate	0.21	0.23	0.51
	4X Rate	0.28	0.30	0.14
	10X Rate	0.28	0.15	0.12
Orelia	1X Rate	0.16	1.00	0.22
	2x Rate	1.00	0.12	1.00
	4X Rate	1.00	0.16	0.12
	10X Rate	0.32	0.16	0.12
Weswood	1X Rate	0.19	0.27	0.50
	2x Rate	0.18	0.35	0.22
	4X Rate	0.40	0.69	0.27
	10X Rate	0.32	0.19	0.21

Table 14 Continued

Soils	Rate	Treatment p-values		
		Mesotrione	Mesotrione + atrazine	Control
Day 42				
Cameron	1X Rate	0.12	0.14	0.14
	2x Rate	0.24	0.22	0.21
	4X Rate	0.37	0.26	0.19
	10X Rate	0.12	0.13	0.16
Darco	1X Rate	0.12	0.13	0.18
	2x Rate	0.27	0.14	0.35
	4X Rate	0.89	0.20	0.15
	10X Rate	0.16	0.82	0.21
Orelia	1X Rate	0.27	0.22	0.27
	2x Rate	0.71	0.12	0.13
	4X Rate	0.50	0.14	0.15
	10X Rate	0.35	0.92	0.50
Weswood	1X Rate	0.27	0.27	0.22
	2x Rate	0.14	0.15	0.15
	4X Rate	0.19	0.12	0.14
	10X Rate	0.57	0.27	0.49
Day 55				
Cameron	1X Rate	0.12	0.77	0.86
	2x Rate	0.12	0.17	0.12
	4X Rate	0.13	0.12	0.37
	10X Rate	0.81	0.31	0.83
Darco	1X Rate	0.71	0.35	0.17
	2x Rate	0.21	0.12	0.35
	4X Rate	0.42	0.15	0.85
	10X Rate	0.53	0.28	0.12
Orelia	1X Rate	0.21	0.12	0.13
	2x Rate	0.13	0.12	0.22
	4X Rate	0.12	0.12	1.00
	10X Rate	0.27	0.23	0.29
Weswood	1X Rate	0.19	0.12	0.14
	2x Rate	0.60	0.92	0.21
	4X Rate	0.12	0.17	0.12
	10X Rate	0.13	0.13	0.39

suggesting that the variances for the two experimental runs were homogeneous. As a result of having homogeneous variances, the data from the two experimental runs was combined for further analysis.

A Fisher's LSD test at the 5% level of significance was conducted to evaluate the treatment and rate effects on soil microbial respiration. Table 15 presents the treatment means (cumulative mg CO_2-C g $soil^{-1}$) and statistical differences observed for the control and mesotrione when applied alone and in combination with atrazine for each sampling day, rate, and soil. Table 16 presents the treatment means and statistical differences for mesotrione when applied alone and in combination with atrazine rates for each sampling day and soil.

Results for the Cameron soil show that, at the 1X rate soil microbial respiration for both the mesotrione and mesotrione treatments were not significantly different from the control. Perhaps for this particular soil, the amount of carbon added in the 1X rate for both treatments was not enough to see a microbial response in terms of respiration. At the 2X rate, significant differences exist at days 16, 24, 32 and 42 where the mesotrione + atrazine was significantly lower than the control at days 16 and 24. Data from the 4X rate (days 16, 24, and 32) and at the 10X rate (day 16) demonstrate significantly lower differences for the mesotrione + atrazine treatment, resulting in lower cumulative mg CO_2-C g $soil^{-1}$ (Table 15, Fig.15).

Table 15 Treatment means of Cumulative mg CO_2-C g $soil^{-1}$ and statistical differences for mesotrione when applied alone and in combination with atrazine for each sampling day, rate, and soil.

		Treatment means (cumulative mg CO_2-C g $soil^{-1}$)		
Soils	Rate	Mesotrione	Mesotrione + atrazine	Control
Day 0				
Cameron	1X Rate	0.014[a]	0.031[a]	0.026[a]
	2x Rate	0.016[a]	0.015[a]	0.018[a]
	4X Rate	0.193[a]	0.014[a]	0.020[a]
	10X Rate	0.017[a]	0.016[a]	0.015[a]
Darco	1X Rate	0.022[a]	0.019 [a]	0.020[a]
	2x Rate	0.025[a]	0.031[a]	0.023[a]
	4X Rate	0.034[a]	0.031[a]	0.030[a]
	10X Rate	0.034[a]	0.032[a]	0.031[a]
Orelia	1X Rate	0.028[a]	0.024[a]	0.016[a]
	2x Rate	0.020[a]	0.022[a]	0.019[a]
	4X Rate	0.026[a]	0.019[a]	0.021[a]
	10X Rate	0.020[a]	0.016[a]	0.015[a]
Weswood	1X Rate	0.031[a]	0.023[b]	0.023[b]
	2x Rate	0.017[a]	0.019[a]	0.016[a]
	4X Rate	0.018[a]	0.024[a]	0.019[a]
	10X Rate	0.022[a]	0.029[ab]	0.029[b]
Day 4				
Cameron	1X Rate	0.050[a]	0.062[a]	0.058[a]
	2x Rate	0.045[a]	0.043[a]	0.054[a]
	4X Rate	0.054[a]	0.040[a]	0.054[a]
	10X Rate	0.048[a]	0.048[a]	0.043[a]
Darco	1X Rate	0.059[a]	0.058[a]	0.077[a]
	2x Rate	0.068[a]	0.102[a]	0.091[a]
	4X Rate	0.074[b]	0.108[a]	0.103[a]
	10X Rate	0.068[a]	0.089[b]	0.090[b]
Orelia	1X Rate	0.051[a]	0.051[a]	0.043[a]
	2x Rate	0.026[a]	0.054[b]	0.045[b]
	4X Rate	0.052[a]	0.044[a]	0.041[a]
	10X Rate	0.045[a]	0.039[a]	0.043[a]
Weswood	1X Rate	0.048[ab]	0.027[b]	0.067[a]
	2x Rate	0.035[a]	0.040[a]	0.060[b]
	4X Rate	0.048[a]	0.042[a]	0.058[a]
	10X Rate	0.048[a]	0.069[b]	0.058[ab]

Table 15 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine	Control
Day 8				
Cameron	1X Rate	0.090[a]	0.091[a]	0.100[a]
	2x Rate	0.081[a]	0.070[a]	0.091[a]
	4X Rate	0.085[a]	0.068[a]	0.091[a]
	10X Rate	0.083[a]	0.082[a]	0.093[a]
Darco	1X Rate	0.083[b]	0.094[ab]	0.124[a]
	2x Rate	0.112[a]	0.155[a]	0.148[a]
	4X Rate	0.123[a]	0.158[a]	0.140[a]
	10X Rate	0.100[a]	0.130[b]	0.126[b]
Orelia	1X Rate	0.076[a]	0.076[a]	0.079[a]
	2x Rate	0.060[a]	0.090[a]	0.068[a]
	4X Rate	0.080[a]	0.083[a]	0.068[a]
	10X Rate	0.078[a]	0.067[a]	0.070[a]
Weswood	1X Rate	0.106 [a]	0.078[a]	0.082[a]
	2x Rate	0.058[a]	0.064[a]	0.086[b]
	4X Rate	0.082[a]	0.095[a]	0.106[a]
	10X Rate	0.082[b]	0.119[a]	0.097[ab]
Day 16				
Cameron	1X Rate	0.147[a]	0.142[a]	0.137[a]
	2x Rate	0.117[ab]	0.092[b]	0.134[a]
	4X Rate	0.134[ab]	0.089[b]	0.148[a]
	10X Rate	0.117[ab]	0.096[b]	0.151[a]
Darco	1X Rate	0.131[a]	0.112[a]	0.192[b]
	2x Rate	0.152[a]	0.237[b]	0.213[ab]
	4X Rate	0.182[a]	0.214[a]	0.205[a]
	10X Rate	0.144[a]	0.179[b]	0.187[b]
Orelia	1X Rate	0.100[a]	0.111[a]	0.106[a]
	2x Rate	0.100[a]	0.133[a]	0.102[a]
	4X Rate	0.111[a]	0.109[a]	0.106[a]
	10X Rate	0.111[a]	0.087[a]	0.107[a]
Weswood	1X Rate	0.181[a]	0.148[ab]	0.116[b]
	2x Rate	0.091[a]	0.097[a]	0.127[b]
	4X Rate	0.157[a]	0.156[a]	0.137[a]
	10X Rate	0.151[a]	0.205[b]	0.127[a]

Table 15 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine	Control
Day 24				
Cameron	1X Rate	0.203[a]	0.171[a]	1.174[a]
	2x Rate	0.175[a]	0.123[b]	0.166[a]
	4X Rate	0.162[ab]	0.119[b]	0.195[a]
	10X Rate	0.158[a]	0.126[a]	0.178[a]
Darco	1X Rate	0.175[ab]	0.122[b]	0.244[a]
	2x Rate	0.256[a]	0.303[a]	0.256[a]
	4X Rate	0.248[a]	0.246[a]	0.251[a]
	10X Rate	0.221[a]	0.218[a]	0.240[a]
Orelia	1X Rate	0.131[a]	0.134[a]	0.135[a]
	2x Rate	0.129[a]	0.161[a]	0.138[a]
	4X Rate	0.135[a]	0.142[a]	0.131[a]
	10X Rate	0.142[a]	0.117[a]	0.127[a]
Weswood	1X Rate	0.223[a]	0.188[ab]	0.145[b]
	2x Rate	0.137[a]	0.134[a]	0.158[a]
	4X Rate	0.214[a]	0.186[a]	0.163[a]
	10X Rate	0.171[a]	0.227[b]	0.157[a]
Day 32				
Cameron	1X Rate	0.231 [a]	0.200[a]	0.202[a]
	2x Rate	0.207[a]	0.152[b]	0.198[ab]
	4X Rate	0.203[a]	0.144[b]	0.209[a]
	10X Rate	0.199[a]	0.153[a]	0.202[a]
Darco	1X Rate	0.189[ab]	0.148[b]	0.272[a]
	2x Rate	0.217[a]	0.355[b]	0.291[ab]
	4X Rate	0.313[a]	0.282[a]	0.277[a]
	10X Rate	0.299[a]	0.267[a]	0.263[a]
Orelia	1X Rate	0.153[a]	0.148[a]	0.147[a]
	2x Rate	0.132[a]	0.191[a]	0.142[a]
	4X Rate	0.140[a]	0.154[a]	0.145[a]
	10X Rate	0.172[a]	0.150[a]	0.140[a]
Weswood	1X Rate	0.264[a]	0.227[ab]	0.179[b]
	2x Rate	0.185[a]	0.152[a]	0.186[a]
	4X Rate	0.249[a]	0.228[a]	0.192[a]
	10X Rate	0.198[a]	0.297[b]	0.190[a]

Table 15 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine	Control
Day 42				
Cameron	1X Rate	0.281[a]	0.234[a]	0.248[a]
	2x Rate	0.261[a]	0.187[b]	0.238[ab]
	4X Rate	0.240[a]	0.178[a]	0.243[a]
	10X Rate	0.246[a]	0.182[a]	0.233[a]
Darco	1X Rate	0.219[ab]	0.173[b]	0.304[a]
	2x Rate	0.236[a]	0.397[b]	0.318[ab]
	4X Rate	0.388[a]	0.320[ab]	0.278[b]
	10X Rate	0.344[a]	0.288[a]	0.302[a]
Orelia	1X Rate	0.190[a]	0.175[a]	0.161[a]
	2x Rate	0.150[a]	0.216[b]	0.162[ab]
	4X Rate	0.155[a]	0.181[a]	0.167[a]
	10X Rate	0.208[a]	0.188[a]	0.154[a]
Weswood	1X Rate	0.298[a]	0.257[ab]	0.207[b]
	2x Rate	0.234[a]	0.168[a]	0.205[a]
	4X Rate	0.313[a]	0.302[ab]	0.217[b]
	10X Rate	0.218[a]	0.364[b]	0.210[a]
Day 55				
Cameron	1X Rate	0.332[a]	0.290[a]	0.310[a]
	2x Rate	0.321[a]	0.237[a]	0.293[a]
	4X Rate	0.293[a]	0.245[a]	0.319[a]
	10X Rate	0.310[a]	0.240[a]	0.282[a]
Darco	1X Rate	0.292[ab]	0.204[b]	0.377[a]
	2x Rate	0.309[b]	0.481[a]	0.409[ab]
	4X Rate	0.459[a]	0.373[a]	0.394[a]
	10X Rate	0.569[a]	0.346[b]	0.346[b]
Orelia	1X Rate	0.236[a]	0.215[a]	0.193[a]
	2x Rate	0.195[a]	0.255[a]	0.188[a]
	4X Rate	0.190[a]	0.218[a]	0.192[a]
	10X Rate	0.238[a]	0.272[a]	0.176[a]
Weswood	1X Rate	0.390[a]	0.323[a]	0.273[a]
	2x Rate	0.303[a]	0.209[a]	0.250[a]
	4X Rate	0.368[a]	0.363[a]	0.289[a]
	10X Rate	0.253[a]	0.528[b]	0.268[a]

Means followed by different letters in a row indicate significant differences between the control and mesotrione treatments when applied alone and in combination with atrazine for each sampling day, rate, and soil.

Table 16 Treatment means and statistical differences for mesotrione when applied alone and in combination with atrazine rates for each sampling day and soil after 55 days of incubation.

		Treatment means (cumulative mg CO_2-C g $soil^{-1}$)	
Soils	Rate	Mesotrione	Mesotrione + atrazine
Day 0			
Cameron	1X Rate	0.014[a]	0.030[a]
	2x Rate	0.168[a]	0.015[a]
	4X Rate	0.193[a]	0.014[a]
	10X Rate	0.017[a]	0.016[a]
Darco	1X Rate	0.022[a]	0.193[a]
	2x Rate	0.025[a]	0.031[b]
	4X Rate	0.034[a]	0.031[b]
	10X Rate	0.034[a]	0.032[b]
Orelia	1X Rate	0.028[a]	0.024[a]
	2x Rate	0.020[a]	0.022[a]
	4X Rate	0.026[a]	0.019[a]
	10X Rate	0.020[a]	0.016[a]
Weswood	1X Rate	0.031[a]	0.023[ab]
	2x Rate	0.017[b]	0.019[b]
	4X Rate	0.018[b]	0.024[ab]
	10X Rate	0.022[b]	0.029[a]
Day 4			
Cameron	1X Rate	0.050[a]	0.062[a]
	2x Rate	0.045[a]	0.043[a]
	4X Rate	0.054[a]	0.040[a]
	10X Rate	0.048[a]	0.048[a]
Darco	1X Rate	0.059[a]	0.058[a]
	2x Rate	0.068[a]	0.102[b]
	4X Rate	0.074[a]	0.108[b]
	10X Rate	0.068[a]	0.089[ab]
Orelia	1X Rate	0.051[a]	0.051[a]
	2x Rate	0.026[b]	0.054[a]
	4X Rate	0.052[a]	0.044[a]
	10X Rate	0.045[a]	0.039[a]
Weswood	1X Rate	0.048[a]	0.027[a]
	2x Rate	0.035[a]	0.040[ab]
	4X Rate	0.048[a]	0.042[b]
	10X Rate	0.048[a]	0.069[c]

Table 16 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine
Day 8			
Cameron	1X Rate	0.090[a]	0.091[a]
	2x Rate	0.081[a]	0.070[a]
	4X Rate	0.085[a]	0.068[a]
	10X Rate	0.083[a]	0.082[a]
Darco	1X Rate	0.083[a]	0.094[a]
	2x Rate	0.112[ab]	0.155[b]
	4X Rate	0.123[b]	0.158[b]
	10X Rate	0.100[ab]	0.130[ab]
Orelia	1X Rate	0.076[a]	0.076[a]
	2x Rate	0.060[a]	0.090[a]
	4X Rate	0.080[a]	0.833[a]
	10X Rate	0.078[a]	0.067[a]
Weswood	1X Rate	0.106[a]	0.078[a]
	2x Rate	0.058[b]	0.064[a]
	4X Rate	0.082[ab]	0.095[ab]
	10X Rate	0.082[ab]	0.119[b]
Day 16			
Cameron	1X Rate	0.147[a]	0.142 [a]
	2x Rate	0.117[a]	0.092[b]
	4X Rate	0.134[a]	0.089[b]
	10X Rate	0.117[a]	0.096[b]
Darco	1X Rate	0.131[a]	0.112[a]
	2x Rate	0.152[a]	0.237[b]
	4X Rate	0.182[a]	0.214[b]
	10X Rate	0.144[a]	0.179[ab]
Orelia	1X Rate	0.100[a]	0.111[a]
	2x Rate	0.100[a]	0.133[a]
	4X Rate	0.111[a]	0.109[a]
	10X Rate	0.111[a]	0.087[a]
Weswood	1X Rate	0.181[a]	0.148[a]
	2x Rate	0.091[b]	0.097[b]
	4X Rate	0.157[a]	0.156[a]
	10X Rate	0.151[a]	0.205[c]

Table 16 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine
Day 24			
Cameron	1X Rate	0.203[a]	0.171[a]
	2x Rate	0.175[a]	0.123[ab]
	4X Rate	0.162[a]	0.119[b]
	10X Rate	0.158[a]	0.126[ab]
Darco	1X Rate	0.175[a]	0.122[a]
	2x Rate	0.256[b]	0.303[b]
	4X Rate	0.248[b]	0.246[bc]
	10X Rate	0.221[ab]	0.218[c]
Orelia	1X Rate	0.131[a]	0.134[a]
	2x Rate	0.129[a]	0.161[a]
	4X Rate	0.135[a]	0.142[a]
	10X Rate	0.142[a]	0.117[a]
Weswood	1X Rate	0.222[a]	0.188[a]
	2x Rate	0.137[b]	0.134[b]
	4X Rate	0.214[a]	0.186[a]
	10X Rate	0.171[ab]	0.227[c]
Day 32			
Cameron	1X Rate	0.231[a]	0.200[a]
	2x Rate	0.207[a]	0.152[a]
	4X Rate	0.203[a]	0.144[a]
	10X Rate	0.199[a]	0.153[a]
Darco	1X Rate	0.189[a]	0.148[a]
	2x Rate	0.217[ab]	0.355[b]
	4X Rate	0.313[c]	0.282[b]
	10X Rate	0.300[ab]	0.267[b]
Orelia	1X Rate	0.153[a]	0.148[a]
	2x Rate	0.132[a]	0.191[a]
	4X Rate	0.140[a]	0.154[a]
	10X Rate	0.172[a]	0.150[a]
Weswood	1X Rate	0.264[a]	0.227[a]
	2x Rate	0.185[a]	0.152[b]
	4X Rate	0.249[a]	0.228[a]
	10X Rate	0.198[a]	0.297[c]

Table 16 Continued

Soils	Rate	Treatment means (cumulative mg CO_2-C g $soil^{-1}$) Mesotrione	Mesotrione + atrazine
Day 42			
Cameron	1X Rate	0.281[a]	0.234[a]
	2x Rate	0.261[a]	0.187[a]
	4X Rate	0.240[a]	0.178[a]
	10X Rate	0.246[a]	0.182[a]
Darco	1X Rate	0.219[a]	0.173[a]
	2x Rate	0.236[a]	0.397[b]
	4X Rate	0.388[b]	0.320[bc]
	10X Rate	0.344[b]	0.288[c]
Orelia	1X Rate	0.190[a]	0.175[a]
	2x Rate	0.150[a]	0.216[a]
	4X Rate	0.155[a]	0.181[a]
	10X Rate	0.208[a]	0.188[a]
Weswood	1X Rate	0.298[a]	0.257[a]
	2x Rate	0.234[a]	0.168[b]
	4X Rate	0.313[a]	0.302[ac]
	10X Rate	0.218[a]	0.364[c]
Day 55			
Cameron	1X Rate	0.332[a]	0.290[a]
	2x Rate	0.321[a]	0.237[a]
	4X Rate	0.293[a]	0.245[a]
	10X Rate	0.310[a]	0.240[a]
Darco	1X Rate	0.292[a]	0.204[a]
	2x Rate	0.309[a]	0.481[b]
	4X Rate	0.459[b]	0.373[bc]
	10X Rate	0.569[b]	0.346[c]
Orelia	1X Rate	0.236[a]	0.215[a]
	2x Rate	0.195[a]	0.255[a]
	4X Rate	0.190[a]	0.218[a]
	10X Rate	0.238[a]	0.272[a]
Weswood	1X Rate	0.390[a]	0.323[a]
	2x Rate	0.303[a]	0.209[b]
	4X Rate	0.368[a]	0.363[a]
	10X Rate	0.253[a]	0.528[c]

Means followed by different letters in a column indicate significant differences between rates for mesotrione treatments when applied alone and in combination with atrazine for each sampling day and soil.

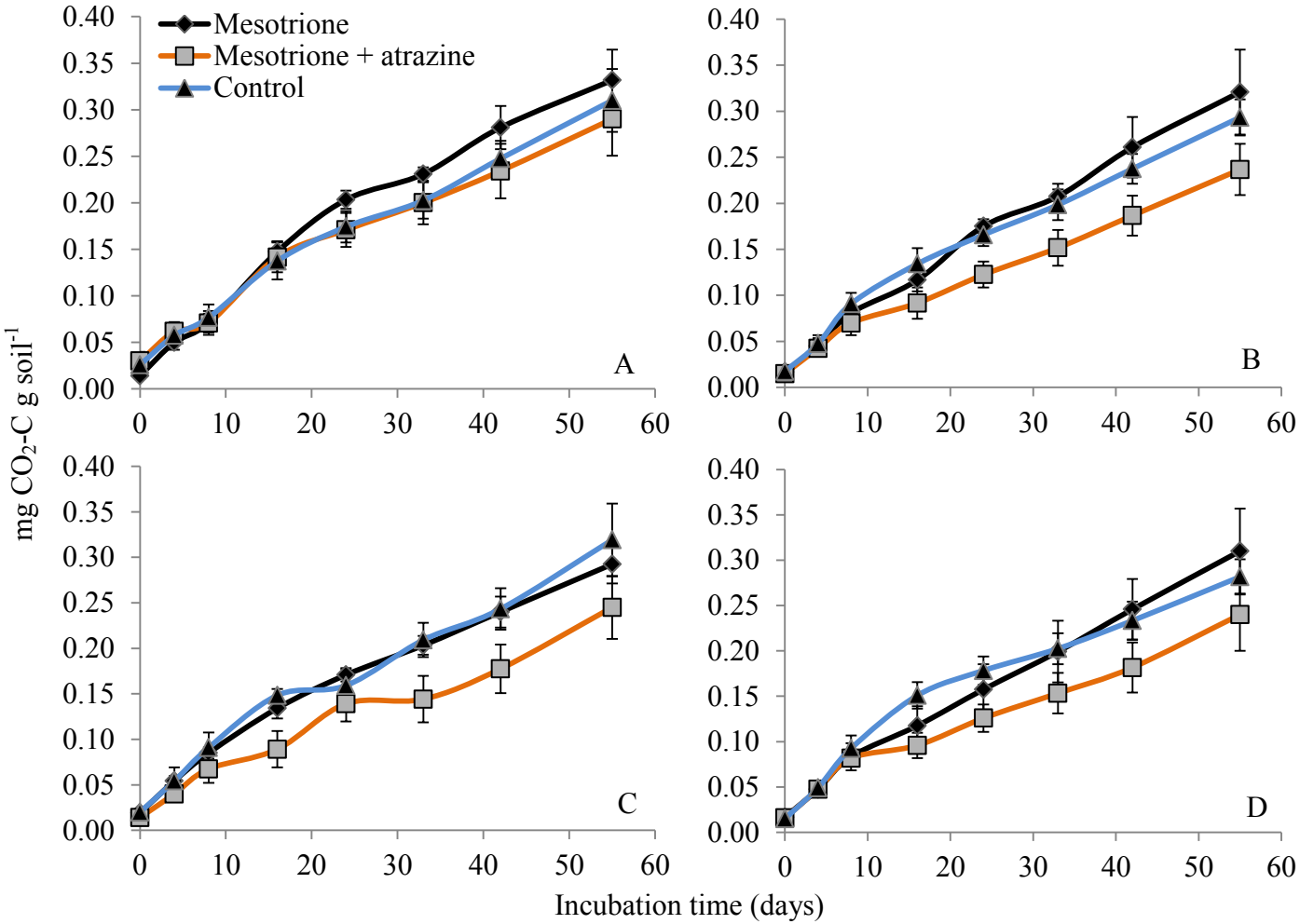

Fig. 15 The effect of four rates of mesotrione (♦) and mesotrione + atrazine (■) treatments (A: mesotrione: 1X, 0.14 µg g^{-1}; B: 2X, 0.28 µg g^{-1}; C: 4X, 0.56 µg g^{-1}; and D: 10X, 1.4 µg g^{-1} A: atrazine: 1X, 0.9 µg g^{-1}; B: 2X, 1.8 µg g^{-1}; C: 4X, 3.6 µg g^{-1}; and D: 10X, 9 µg g^{-1}) on microbial respiration for the Cameron soil. Error bars represent the standard error of the mean.

In terms of the mesotrione treatment, similar results have been observed by Crouzet et al. (2010) where the response of soil microbial communities to the addition of the herbicide mesotrione was evaluated. The soil used in that study was similar in texture (clay loam) than the Cameron soil. They found that when mesotrione was applied at the 1X rate, it did not have an effect on soil microbial activity, but when the rates were increased, significantly higher microbial activity was observed. Although the mesotrione treatment was not significantly different from the control in all rates in the Cameron soil in my study, it was significantly higher than the mesotrione + atrazine treatment for the 2X rate (days 24, 32, and 42) and for the 10X rate (day 42), evolving higher cumulative mg CO_2-C g $soil^{-1}$. Crouzet et al. (2010) also found that the increase in soil microbial activity was not only as a result of an increase in application rate but also herbicide exposure time. This trend is also observed in this study, significant differences between the mesotrione and mesotrione + atrazine treatments were not observed until days 24 and day 42 for the 2X and 10X rate, respectively. They predict that the increased microbial activity due to increased exposure time could be a result of the growth of resistant populations of microorganisms feeding of the dead biomass from sensitive microorganisms affected by the herbicide treatment.

The data obtained for this soil indicates that the addition of atrazine in the mesotrione + atrazine treatments inhibit soil respiration. A study conducted by Accinelli et al. (2002), found that the addition of pure atrazine at 2 μg g $soil^{-1}$ caused no significant differences in microbial respiration but at 20 μg g $soil^{-1}$, a stimulation of soil microbial activity occurred. When they used 200 μg g^{-1} it resulted in a significant

decrease in soil microbial activity. In this study, at the 10X rate atrazine was added at 9 $\mu g\ g^{-1}$, and an inhibition of soil respiration was observed suggesting that an inhibitory effect to soil microbial respiration is caused when atrazine is added in combination with the mesotrione. Furthermore, it was observed in Chapter III, that the when atrazine was added in combination with mesotrione, mesotrione degradation was inhibited at 30 and 60 days (Fig.8). If an inhibition is caused by this treatment combination due to a toxic effect, this could explain the reduced respiration rates and increased persistence observed in the degradation study (Chapter III). Again, if there is a toxic effect, it does not happen immediately after the addition of the herbicide treatments but after continued exposure (after day 16 in respiration study).

The other objective of this study was to investigate if there is a rate effect on soil microbial respiration. For the Cameron soil, no significant differences were observed for any rate at any days for the mesotrione treatment. In the mesotrione + atrazine treatment, a rate effect was observed at days16 and 24 where the 1X rate differed from all rates and the 4X rate differed from the 1X rate, respectively (Table 16, Figs.16 and 17).

Results for the Darco soil show that at the 1X rate, significant differences were observed after 4 days of incubation (days 8, 16, 24, 32, 42, and 55), where the mesotrione + atrazine treatment is inhibiting microbial respiration (days 16 to 55) and the mesotrione treatment also inhibited microbial respiration at days 8 and 16. At 2X the rate, significant differences were observed at days 16, 32, 42, and 55 between the mesotrione and mesotrione + atrazine treatments, where greater microbial respiration was seen in the mesotrione + atrazine treatment and lower microbial respiration was seen

in the mesotrione treatment but neither treatment was significantly different from the control. At the 4X rate, significant differences were not observed between the three treatments with the exception of day 4 (significantly lower) and day 42 (significantly higher), where differences in the control and mesotrione treatment were observed. At the 10X rate, significant differences were observed at days 4, 8, 16, and 55 between the mesotrione treatment and the control, resulting in a 65% increase in microbial respiration for the mesotrione treatment (Table 15, Fig. 18).

The data suggests that the addition of atrazine in the mesotrione + atrazine treatments caused an inhibitory effect on respiration for the 1X rate from days 16 to 55. The mesotrione treatment also caused an inhibitory effect for the 1X rate at days 8 and 16. Unlike the results seen in the Cameron soil and the study conducted by Crouzet et al. (2010), a negative impact on respiration is observed at the 1X rate. The texture of the Darco soil could explain why an effect on soil microbial activity was observed at the 1X rate. The Darco soil is has the highest percent sand and lowest percent clay. Pesticides have been identified to absorb less in coarse-textured soils with low amounts of organic matter (Stougaard et al. 1990; Peter and Weber 1985), causing the microorganisms to be directly exposed to the herbicide. It is still possible that a toxic effect from the treatment combination of mesotrione + atrazine is occurring for the 1X rate. In Chapter III, it was observed that the addition of atrazine in the mesotrione + atrazine treatment inhibited degradation at 60 days (Fig.9). As in the Cameron soil, this potential toxic effect could explain the reduced respiration rates and increased persistence observed in the degradation study (Chapter III).

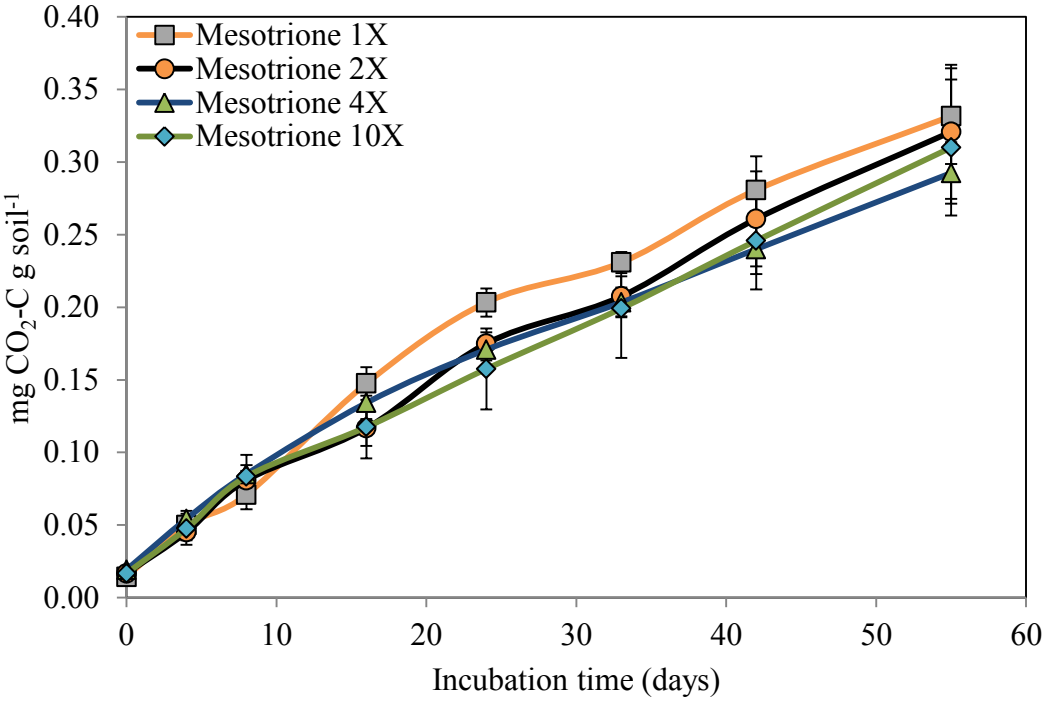

Fig. 16 Soil microbial respiration from four rates of mesotrione (1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1})for the Cameron soil. Error bars represent the standard error of the mean.

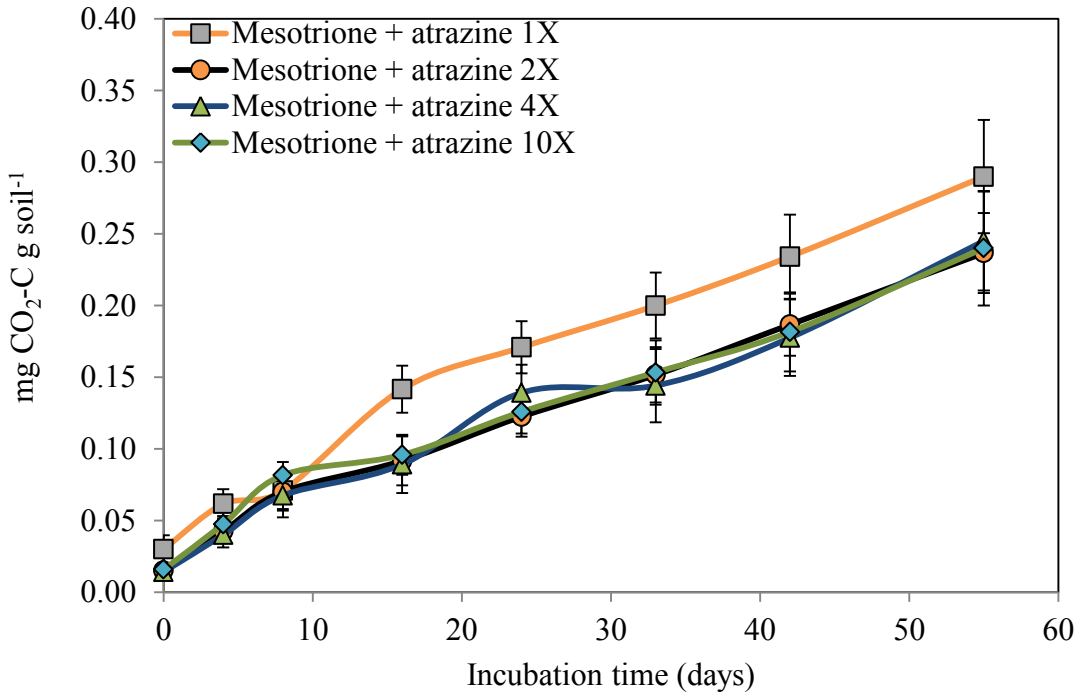

Fig. 17 Soil microbial respiration from four rates of mesotrione + atrazine (mesotrione: 1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}; atrazine: 1X, 0.9 µg g^{-1}; 2X, 1.8 µg g^{-1}; 4X, 3.6 µg g^{-1}; and 10X, 9 µg g^{-1}) for the Cameron soil. Error bars represent the standard error of the mean.

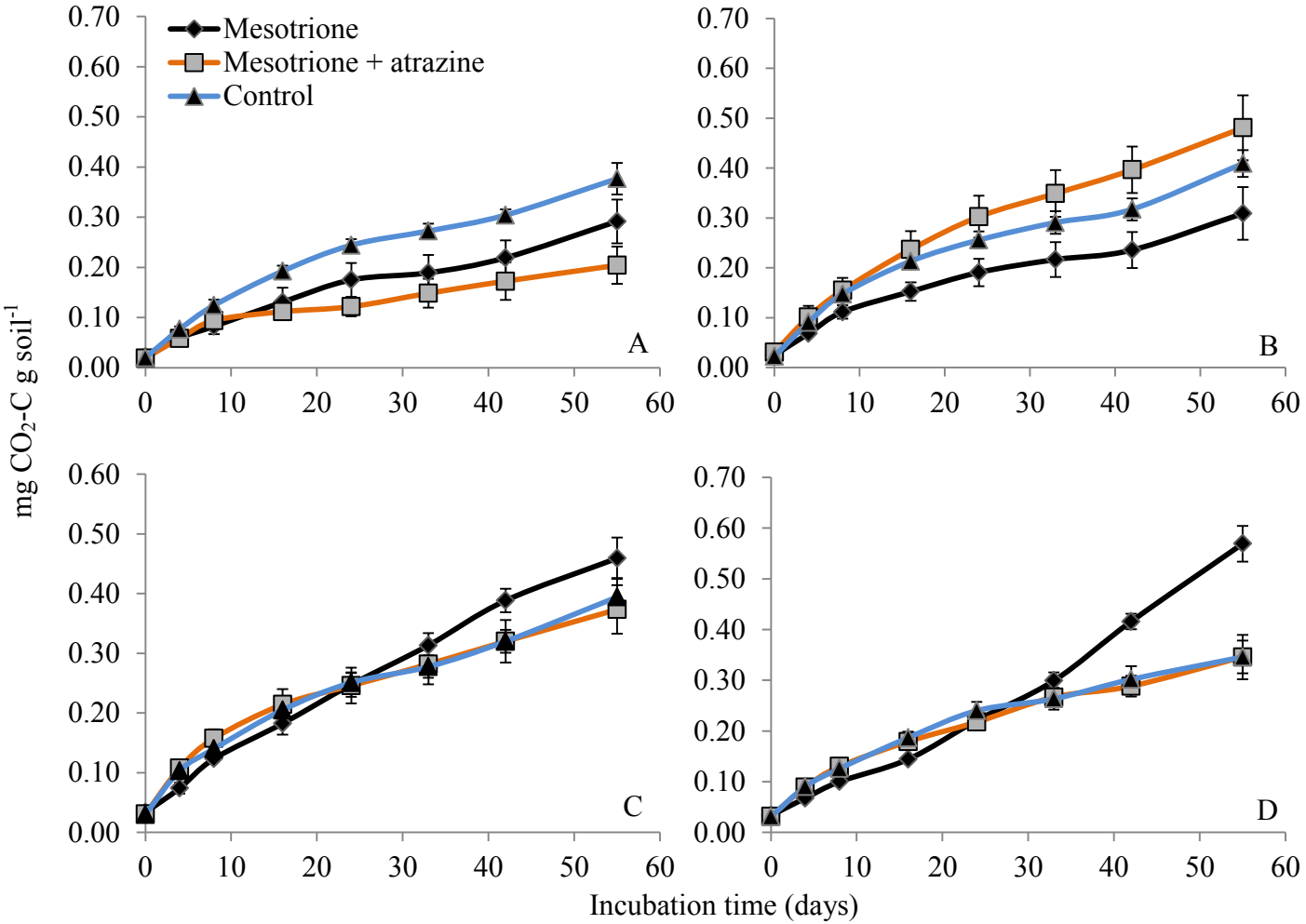

Fig. 18 The effect of four rates of mesotrione (♦) and mesotrione + atrazine (■) treatments (A: mesotrione: 1X, 0.14 µg g^{-1}; B: 2X, 0.28 µg g^{-1}; C: 4X, 0.56 µg g^{-1}; and D: 10X, 1.4 µg g^{-1}; A: atrazine: 1X, 0.9 µg g^{-1}; B: 2X, 1.8 µg g^{-1}; C: 4X, 3.6 µg g^{-1}; and D: 10X, 9 µg g^{-1}) on microbial respiration for the Darco soil. Error bars represent the standard error of the mean

Although this behavior is not observed in the 2X rate or the 10X rate, significant differences from the control were not observed. At the 10X rate, significantly higher differences were observed between mesotrione treatment and the control at days 4, 8, 16, and 55, resulting in a 65% increase in microbial respiration by day 55.

The 65% increase in microbial respiration for the mesotrione treatment is not expected to be a result of the amount of carbon added (6.93×10^{-4} mg C g $soil^{-1}$) with the addition of the mesotrione treatment. Moreno et al. (2007) has observed an increase in microbial activity only after lengthy incubation, after 45 days with atrazine. The same was observed by Crouzet et al. (2010) where an increase in microbial activity was seen with mesotrione at higher herbicide doses and increases exposure time. Perhaps, the same is occurring with mesotrione and this is the reason why significant differences are observed at day 42 at the 4X and day 55 at the 10X. Moreno et al. (2007) explains that it is possible that the microbial activity increased as an adaptation to the stress caused by the herbicide being added. It is also possible that the addition of the mesotrione treatment at the 10X rate could have had an indirect effect and stimulated saprophytic communities to feed on the dead biomass from sensitive microorganisms and that could be the increased microbial respiration that is observed (Crouzet et al. 2010).

The possibility exists that herbicides could impair soil microalgae and cyanobacteria as a result of their metabolic pathways being similar to target weed species (Megharaj et al. 1999; Zancan et al. 2006). Mesotrione acts by inhibiting the HPPD enzyme (Mitchell et al. 2001) in target weed species, the same enzyme exists in microalgae and cyanobacteria (Trebs et al. 2004). Crouzet et al. 2013, conducted a study

to determine the dose-dependent effect of mesotrione and Callisto® on soil photosynthetic microorganisms. They found that no effect was detected in soils treated with 1X the rate for either formulation. At the 10X rate, only Callisto® treatment induced significant decreases in photosynthetic biomass. When they used the 100X rate, both formulations caused a strong negative impact on soil chlorophyll concentrations and cyanobacterial genetic structure and diversity.

Perhaps in the Darco soil, at the higher rates (4X and 10X), the mesotrione treatment is negatively impacting the soil cyanobacterial communities, and the 65% increase in microbial respiration observed at the 10X rate could be a result of other soil microorganisms feeding off the dead biomass of the cyanobacteria. If the mesotrione treatment is negatively impacting the cyanobacterial communities in the Darco soil at lower rates (4X and 10X the rate) than in the soil used in the study conducted by Crouzet et al. 2013, it can be attributed to differences in the soil characteristics.

For the rate effect in the Darco soil, significant differences were observed for days 8, 24, 32, 42, and 55 for the mesotrione treatment. More cumulative mg CO_2-C g $soil^{-1}$ was evolved for the 10X and 4X rate while the 2X and 1X rate were lower. For the rate effect in the mesotrione + atrazine treatment, significant differences were observed for all sampling days where the 1X rate was significantly lower than the 2X and 4X rate (Table 16, Figs. 19 and 20).

Results for the Orelia soil show no significant differences among treatments in the 1X, 4X, and 10X rates. At 2X rate, significant differences were not observed between the three treatments with the exception of day 4 and day 42, where significantly

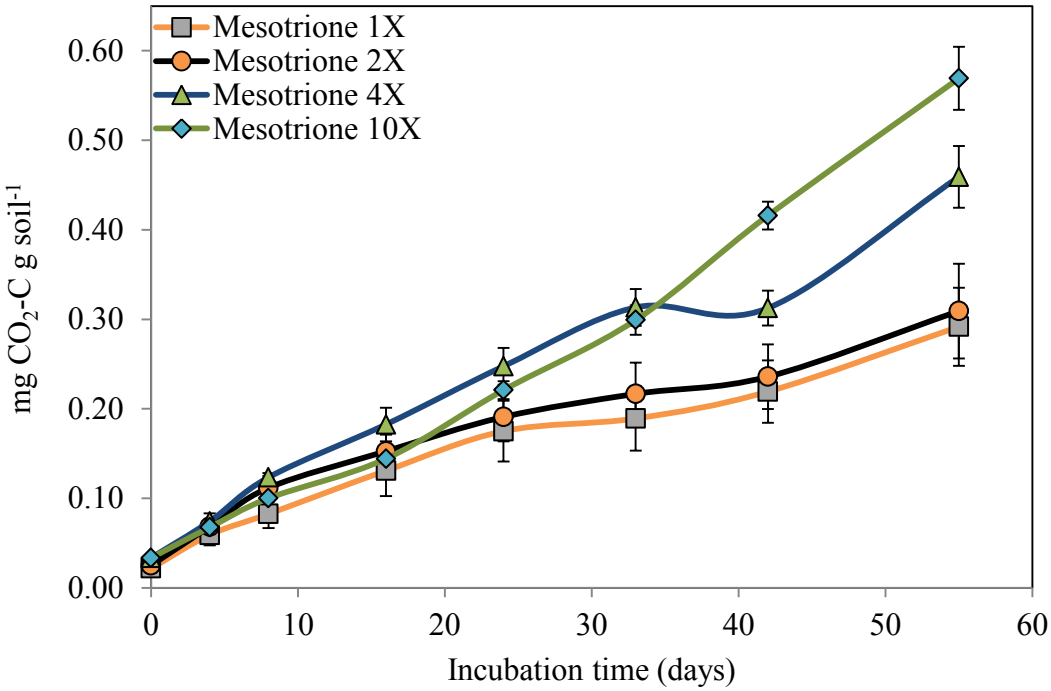

Fig. 19 Soil microbial respiration from four rates of mesotrione (1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}) for the Darco soil. Error bars represent the standard error of the mean

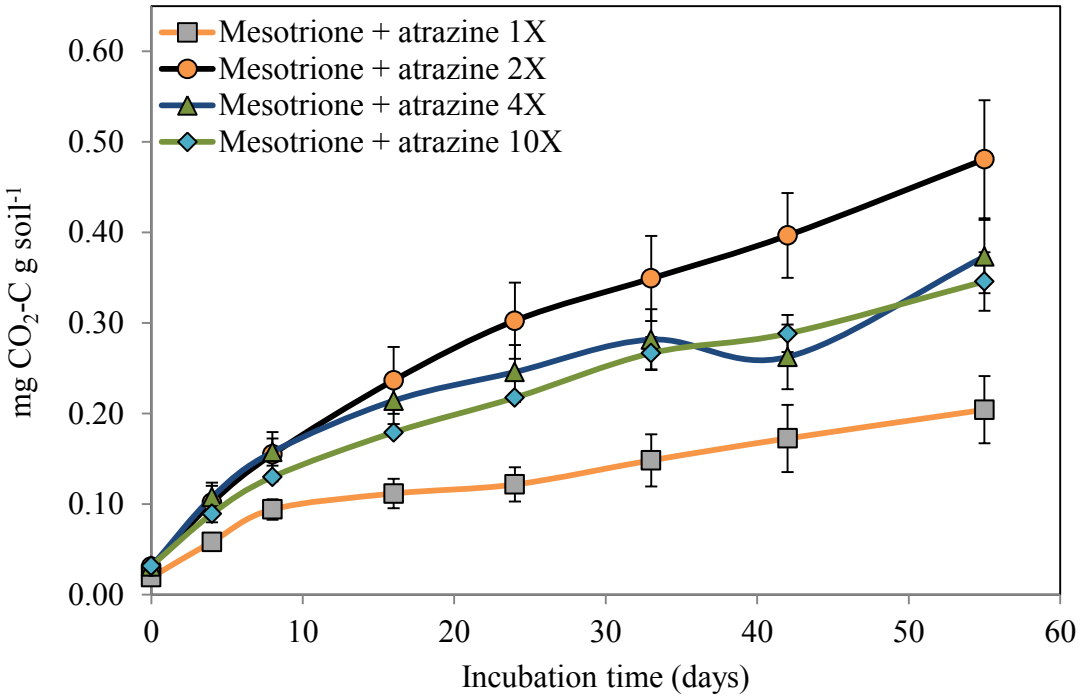

Fig. 20 Soil microbial respiration from four rates of mesotrione + atrazine (mesotrione: 1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}; atrazine: 1X, 0.9 µg g^{-1}; 2X, 1.8 µg g^{-1}; 4X, 3.6 µg g^{-1}; and 10X, 9 µg g^{-1}) for the Darco soil. Error bars represent the standard error of the mean

lower differences from the control were observed for the mesotrione treatment and significantly higher differences from the control and mesotrione + atrazine treatment were observed, respectively (Table 15, Fig. 21). In Chapter III, it was observed that mesotrione and the addition of atrazine in the mesotrione + atrazine treatment had no significant effect on soil degradation (Fig.10). The data obtained from the degradation study also suggests that minimal mesotrione degradation occurred through the duration of the study in that soil. It is possible that the soil microbial populations present in the Orelia soil could have been unaffected by the addition of the herbicides and have continued to degrade the native organic matter in the soil, accounting for the observed mg CO_2-C g $soil^{-1}$ evolved. Perhaps the soil microbial populations in that soil did not possess the enzyme systems necessary to degrade the added herbicides, causing no significant differences observed in microbial respiration between the treatments. Batisson et al. 2009 conducted a study to isolate and characterize mesotrione-degrading *Bacillus* sp. from soil. They found that the bacterial strain Mes16 (Accession number EU864321) was not able to degrade mesotrione while Mes11 (Accession number EU864320) was able to degrade mesotrione within the first 24 hours of incubation. They discuss how certain strains of bacteria may require a synergistic interaction with other bacteria to degrade mesotrione while others do not. It is also possible that the soil bacteria found in the Orelia soil did have the necessary enzymes necessary to degrade the added herbicides but in order to do so, a synergistic interaction with other bacteria that may not have been present needed to take place for mesotrione degradation to occur, and to observe significant differences in microbial respiration between the treatments.

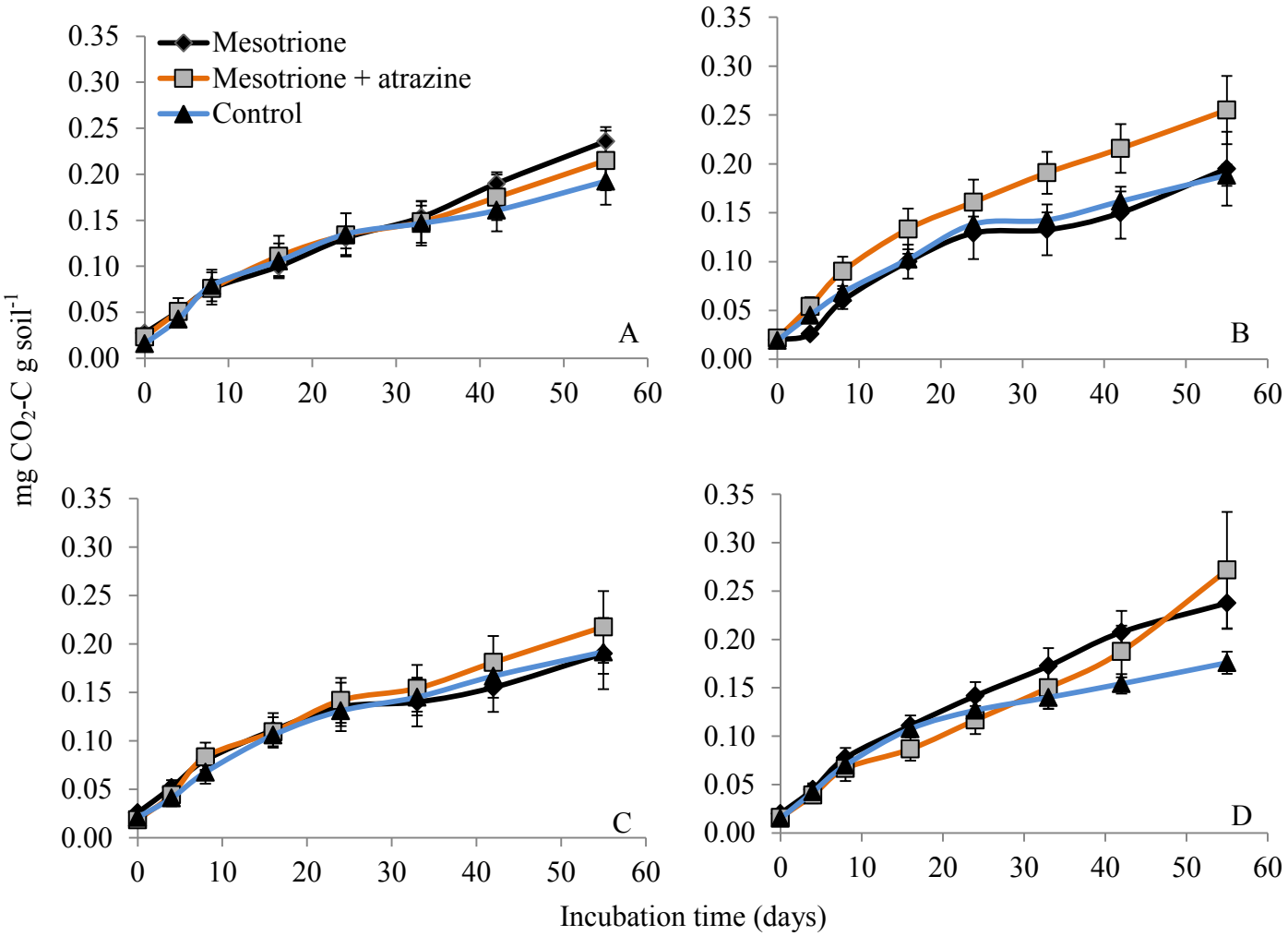

Fig. 21 The effect of four rates of mesotrione and mesotrione + atrazine treatments (A: mesotrione: 1X, 0.14 µg g^{-1}; B: 2X, 0.28 µg g^{-1}; C: 4X, 0.56 µg g^{-1}; and D: 10X, 1.4 µg g^{-1}; A: atrazine: 1X, 0.9 µg g^{-1}; B: 2X, 1.8 µg g^{-1}; C: 4X, 3.6 µg g^{-1}; and D: 10X, 9 µg g^{-1}) on microbial respiration for the Orelia soil. Error bars represent the standard error of the mean.

For the rate effect in the Orelia soil, significant differences were not observed for the mesotrione treatment with the exception of day 4, where the 2X the rate was significantly lower than the other treatments. No significant differences were observed for the mesotrione + atrazine treatment (Table 16, Figs. 22 and 23).

Results for the Weswood soil show that at 1X the rate, significant differences were observed in all days with the exception of days 8 and 55. A slight toxic effect by the addition of atrazine in the mesotrione + atrazine treatment is observed at the 1X rate only at day 4. This was the only soil where the mesotrione treatment evolved more cumulative mg CO_2-C g $soil^{-1}$ from days 16 to 42 at the 1X rate. At 2X the rate, significant differences are only observed at days 4, 8, and 16, where both the treatment means of the mesotrione and mesotrione + atrazine are different from the control, reflecting an inhibition on soil microbial respiration for both treatments. At 4X rate, significant differences were not observed between the three treatments with the exception of day 42, where differences in the control and mesotrione treatment were observed. Significant differences between the three treatments were observed throughout the whole incubation period for the 10X rate, resulting in a 97% increase in microbial respiration for the mesotrione + atrazine treatment (Table 15, Fig. 24). The data indicates that for this soil at lower herbicide application rates (1X and 2X rate) the mesotrione treatment was significantly different from the control, stimulating microbial respiration but at a higher rate 10X rate, the mesotrione + atrazine treatment is the treatment that stimulates microbial respiration. It is possible that a similar situation is occurring as it

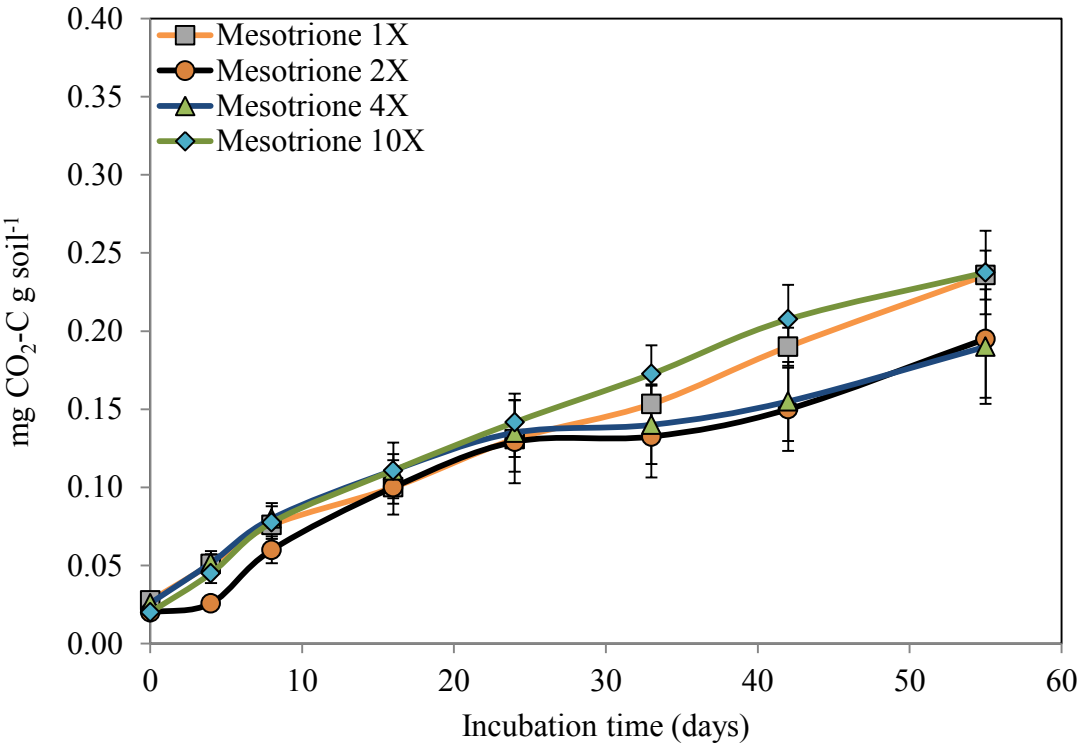

Fig. 22 Soil microbial respiration from four rates of mesotrione (1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}) for the Orelia soil. Error bars represent the standard error of the mean.

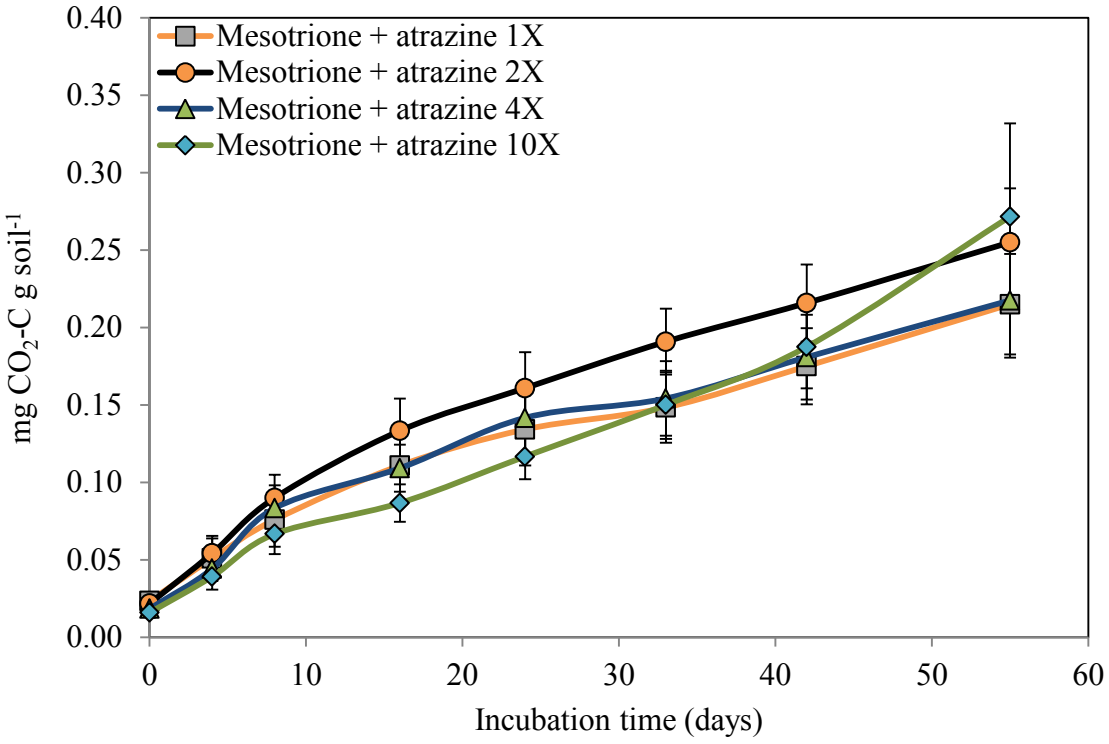

Fig. 23 Soil microbial respiration from four rates of mesotrione + atrazine (mesotrione: 1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}; atrazine: 1X, 0.9 µg g^{-1}; 2X, 1.8 µg g^{-1}; 4X, 3.6 µg g^{-1}; and 10X, 9 µg g^{-1}) for the Orelia soil. Error bars represent the standard error of the mean.

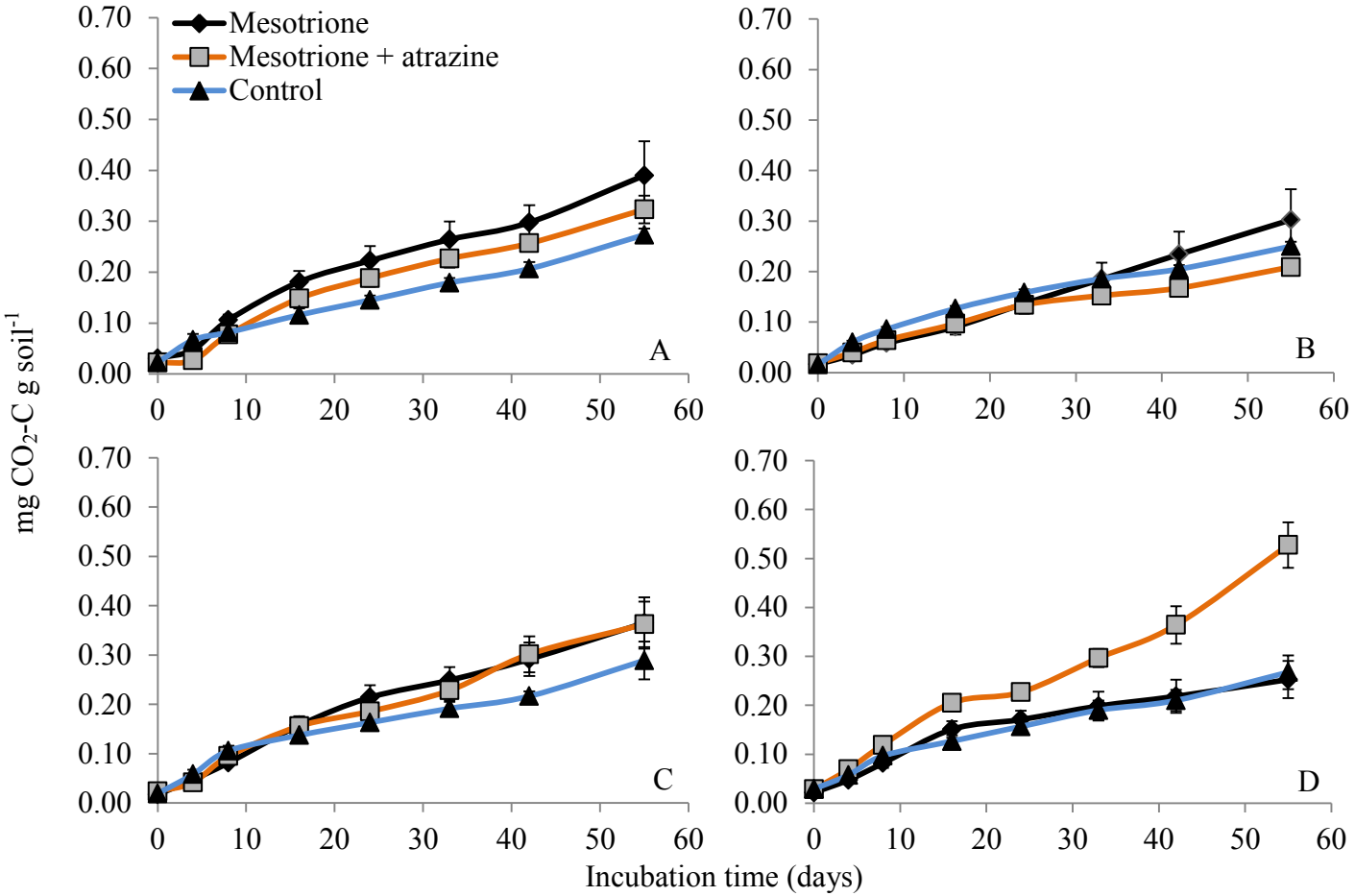

Fig. 24 The effect of four rates of mesotrione (♦) and mesotrione + atrazine (■) treatments (A: mesotrione: 1X, 0.14 µg g^{-1}; B: 2X, 0.28 µg g^{-1}; C: 4X, 0.56 µg g^{-1}; and D: 10X, 1.4 µg g^{-1}; A: atrazine: 1X, 0.9 µg g^{-1}; B: 2X, 1.8 µg g^{-1}; C: 4X, 3.6 µg g^{-1}; and D: 10X, 9 µg g^{-1}) on microbial respiration for the Weswood soil. Error bars represent the standard error of the mean.

did in the Darco soil and what occurred in the study by Moreno et al. (2007) and Crouzet et al. (2010). That after lengthy incubation microbial activity increases due to an adaptation to the stress caused by the herbicide being added. A possible explanation as to why we would see this increase in microbial respiration for the mesotrione + atrazine treatment and not the mesotrione treatment as observed in the Darco soil, is that differences among soil texture, soil organic matter, and even soil microbial populations exist between these two soils. Again, it is possible that the addition of the mesotrione + atrazine treatment at the 10X rate could have had an indirect effect and stimulated saprophytic communities and the increased respiration seen in that treatment is from those microorganisms feeding on the dead biomass. For the rate effect in the Weswood soil, there were significant differences observed for both the mesotrione and mesotrione + atrazine treatments between the rates. In the mesotrione treatment, rate 1X is significantly different than 2X at days 8, 16, and 24, where the 1X rate stimulated more microbial respiration. In the mesotrione + atrazine treatment there was not a clear effect seen on herbicide rate except for the 10X rate at days 4, 16, 24, 32, and 55 which is the rate responsible for stimulating more microbial respiration (Table 15, Fig.25 and 26).

This study focused on analyzing the unknown effects on soil microbial respiration by mesotrione and mesotrione + atrazine treatments in soil. To our knowledge, this is the first study using mesotrione + atrazine mixture, despite the fact that the combination is used to treat undesirable weeds in the U.S. Understanding the soil microbial respiration effects of these treatment combinations is important to obtain a better understanding of the potential effects.

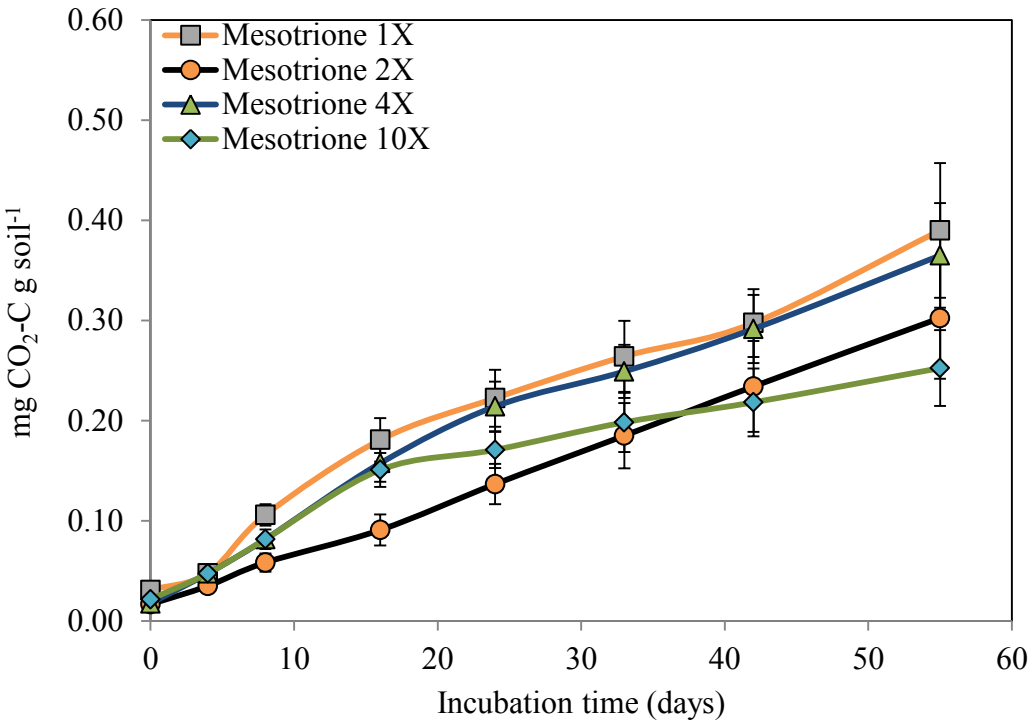

Fig. 25 Soil microbial respiration from four rates of mesotrione (1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}) for the Weswood soil. Error bars represent the standard error of the mean

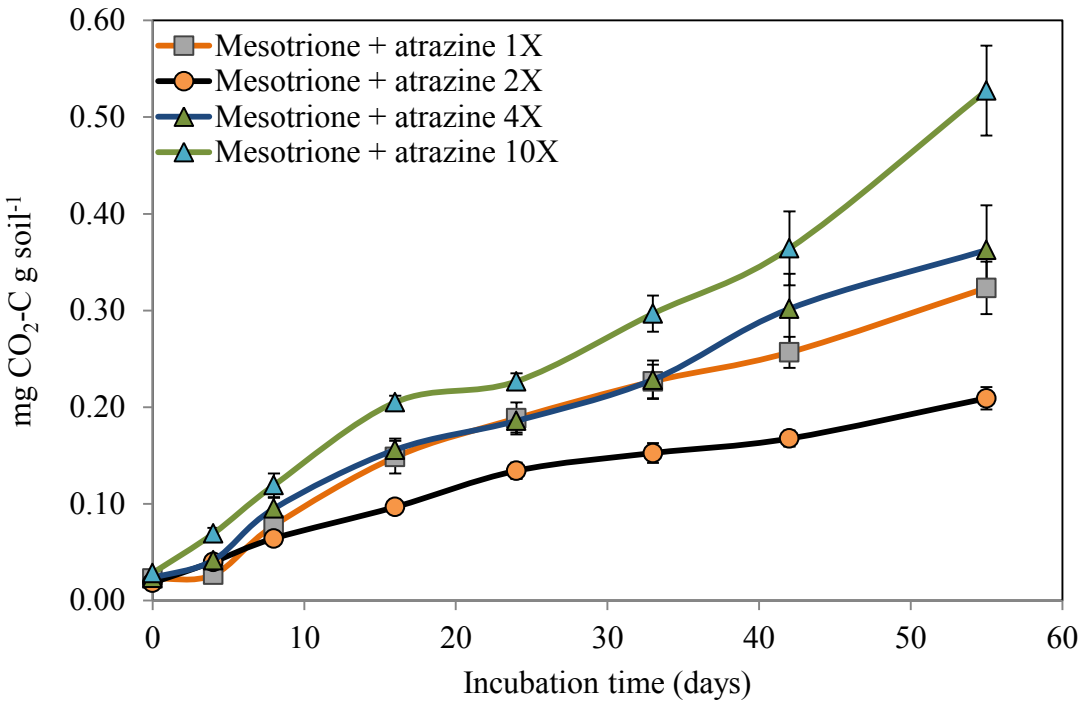

Fig. 26 Soil microbial respiration from four rates of mesotrione + atrazine (mesotrione: 1X, 0.14 µg g^{-1}; 2X, 0.28 µg g^{-1}; 4X, 0.56 µg g^{-1}; and 10X, 1.4 µg g^{-1}; atrazine: 1X, 0.9 µg g^{-1}; 2X, 1.8 µg g^{-1}; 4X, 3.6 µg g^{-1}; and 10X, 9 µg g^{-1}) for the Weswood soil. Error bars represent the standard error of the mean.

The results from this study suggest that mesotrione treatments could have an impact on microbial respiration. It was observed that at the 1X rate in the Darco soil, the mesotrione treatment inhibited microbial respiration (days 8 and 16) and at the 4X rate (day 4). At the 10X rate the mesotrione inhibition of microbial respiration was observed at days 4, 8, and 16. In the Orelia soil, mesotrione inhibited microbial respiration at the 4X rate for day 4. In the Weswood soil, mesotrione inhibited microbial respiration at the 2X rate for days 4, 8, and 12. Furthermore, the mesotrione treatment was found to also stimulate microbial respiration at the 10X rate in the Orelia soil.

The results also suggest that the addition of atrazine in the mesotrione + atrazine could also have an impact on microbial respiration. It was observed that in the Cameron soil the addition of mesotrione + atrazine treatments inhibited microbial respiration for the 2X rate (days 16 and 24) for the 4X rate (days 16, 24, and 32) and the 10X rate (day 16). In the Darco soil, the mesotrione + atrazine treatment inhibited microbial respiration for the 1X rate at days 16 to 55. In the Weswood soil the mesotrione + atrazine treatment inhibited microbial respiration at the 1X rate at day 4. At the 2X rate the mesotrione + atrazine treatment inhibited microbial respiration at days 4, 8, and 16. At the 10X rate the mesotrione + atrazine treatment inhibited microbial respiration at days 16, 24, 32, 42, and 55.

The effect of rates on soil microbial respiration was less evident. A study conducted by Haney et al. (2000) observed that cumulative soil carbon clearly increased with increasing glyphosate rates. This trend was not observed in this study and differences were observed between rates for the four soils, occurring at various time

intervals that did not allow for a distinct relationship between application rates and evolved cumulative mg CO_2-C g $soil^{-1}$. No general trend of microbial responses can be inferred regarding application rate from this study.

The results obtained in this study show that mesotrione and mesotrione + atrazine treatments had the potential to inhibit soil microbial respiration, and on a few instances stimulated microbial respiration. It was not clear if the microbial respiration inhibition/stimulation observed was as a result of combined effects of the herbicides, differences in soil characteristics, and microbial populations present. It is also possible that the differences observed between the soils and application rates could be a result of the history of the soil, rather than the recent treatment of mesotrione and mesotrione + atrazine. Knowing that pesticide mixtures are a current trend in agricultural practices, it is essential that their impact be investigated with more sensitive methodologies, and focus on specific microbial communities to obtain an overall understanding of their potential impact.

CHAPTER V

CONCLUSIONS

Pesticides are a critical component of agricultural systems in the U.S. and many other parts of the world. Concerns regarding their continued use are mainly due to negative associations made between these chemicals and deteriorating environmental, health, and water quality conditions. Although new developments, such as organic (chemical-free) agriculture have taken place, the continued use of agricultural chemicals is expected in order to meet the food demands of a growing world population. Researchers are constantly improving pesticides to possess properties that will help reduce adverse effects and protect the environment. This research focused on the herbicide mesotrione (2-[4-(methysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), a member of the triketone chemical family. An extraction method using ASE was developed for mesotrione in four soils with varying physical and chemical characteristics to reduce solvent waste and improve extraction time to allow for rapid quantification of the herbicide. Later, that method was used to evaluate the impact of combined atrazine application on mesotrione degradation in soil. This study suggested that mesotrione + atrazine herbicide mixtures have the potential to decrease mesotrione degradation in soils. However, it remained unclear whether the reduced degradation was due to the combined impacts of the herbicides, varying soil characteristics, and/or the soil microbial populations present in each soil. Lastly, the effect of mesotrione, mesotrione + atrazine treatments and application rates on soil microbial respiration was evaluated. The results suggested that both the mesotrione and mestotrione + atrazine treatments have the

potential to inhibit microbial respiration. These impacts were observed at several incubation time periods and rates for some soils. Although rate effects on microbial respiration occurred, a trend was not observed in this study. It would be beneficial to conduct more research to determine what specific alterations in the soil microbial populations caused the changes observed in soil microbial respiration.

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